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## **SCIENTIFIC REPORT submitted to EFSA**

### **Scientific review on mosquitoes and mosquito-borne diseases<sup>1</sup>**

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# **Scientific reviews on Classical Swine Fever (CSF), African Swine Fever (ASF) and African Horse Sickness (AHS), and evaluation of the distribution of arthropod vectors and their potential for transmitting exotic or emerging vector-borne animal diseases and zoonoses**



## **Scientific review on mosquitoes and mosquito-borne diseases**

**CFP/EFSA/AHAW/2007/02**





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## INTRODUCTION

During the past 25 years, there has been a dramatic emergence/resurgence of epidemic vector-borne diseases affecting both humans and domestic animals (Gubler, 2002). In most cases, sociodemographic changes (e.g., urbanization, increase of population density, poor housing...), drug resistance and anthropogenic environmental (e.g., deforestation, new dams and irrigation systems...) modifications appear to be the main factors responsible (Martens, 2000; Rogers and Randolph, 2000; Reiter, 2001; Gubler, 2002; Poncon *et al.*, 2007). Nevertheless, strict emergences could occur without apparent cause, such as the introduction and spread of West Nile fever in North America. This kind of events is more likely to happen due to the increase of global trade and people travelling. Even if such emergences remain quite impossible to predict, listing potential vectors of arboviruses in Europe could allow risk anticipation.

The aim of the workpackage is to prepare a scientific review of the latest knowledge on the biology and ecology of *Culex* and *Aedes* species as vectors of the following diseases: Rift Valley fever, Eastern equine encephalitis (EEE), Western equine encephalitis (WEE), Venezuelan equine encephalitis (VEE), Japanese encephalitis, Chikungunya fever, Yellow fever, Dengue and West Nile fever. The epidemiology and main outbreaks of each disease are briefly described.

The first step was to determine vectors of the above diseases in the aim to assess the risk for European countries. Criteria for the recognition of a vector are (WHO, 1961):

- 1) recovery of virus from wild-caught specimens free from visible blood;
- 2) demonstration of ability to become infected by feeding on a viraemic vertebrate host or an artificial substitute;
- 3) demonstration of ability to transmit biologically by bite;
- 4) accumulation of field evidence confirming the significant association of the infected arthropods with the appropriate vertebrate population in which disease or infection is occurring.

Detecting virus in a pool of mosquitoes collected in nature provide an excellent indication that the species could be involved in the transmission cycle of the virus. Nevertheless, detecting virus from only a single pool or even a small number of pools of a species may not implicate that species in the transmission process (Turell *et al.*, 2002). Thus, only **repeated** recoveries of virus from field-collected individuals are considered a proof of potential involvement of a species in virus transmission. Moreover, virus isolation is a better indicator than genome detection, because it proves that insects could carry live virus.

It has been shown that vector competence can change depending on the virus strain and mosquito populations or colonization (Vazeille-Falcoz *et al.*, 1999; Vazeille *et al.*, 2003). Thus, assessment of vector competence should be carried out with a local virus strain and the local mosquito population, or at least, with virus strain with low passage history and with field mosquito populations (or first generation of colonized species). A species should only be considered competent only if females

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can transmit the virus by bite (or at least the virus is recovered from saliva) after the arthropod has fed on a viremic host (or at least after been orally exposed to the virus).

These criteria were used to analyze reports of virus isolations from field-collected mosquitoes and vector competence studies. Then, for each disease, suspected/potential/proven vectors were listed following the recommendations of the World Health Organization (WHO, 1967)<sup>1</sup>. A literature review was focused on proven, potential and suspected vectors which are present in Europe, describing the bio-ecology (seasonality, breeding sites, and feeding habits) of each species. Then, we described the available European surveillance systems when available, the possible control methods and the distribution of each species.

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<sup>1</sup> Suspected vectors fulfil only one of the criteria; potential vectors pass the test of natural infection and experimental transmission; and confirmed vectors fulfil all the conditions.



# 1. IDENTIFICATION OF SUSPECTED/POTENTIAL/PROVEN VECTOR OF SOME ARBOVIROSES IN EUROPE

## 1.1. RIFT VALLEY FEVER

Rift Valley Fever (RVF) is a disease of domestic ruminants, caused by an arbovirus belonging to the *Phlebovirus* genus (*Bunyaviridae* family). It produces high mortality rates in newborn ruminants, especially sheep and goats, and abortion in pregnant animals. It is a zoonotic disease. Human infection by the Rift Valley fever virus (RVFV) may result from direct and close contact with viraemic animals, and through direct contact with their secretions and excretions during caretaking, and with their carcasses and organs including offal, during autopsy, slaughtering and butchering. Until 1975, RVF was regarded as an African that affects only animals. Human cases were rare and with mild clinical manifestations. In 1975, a severe outbreak affecting humans and ruminants occurred in South Africa (Mc Intosh and Jupp, 1981). One of the most noticeable outbreak occurred in East Africa in December 1997, when unexplained human deaths were reported in the North Eastern Province of Kenya and southern Somalia. This epidemic was considered to be the most devastating in the region (Woods *et al.*, 2002). In September 2000, RVF was detected for the first time outside the African continent, in Saudi Arabia and Yemen, leading to human deaths and major losses in livestock populations (Ahmad, 2000). In 2006-2007 an outbreak was declared in Kenya (CDC, 2007), then latter affected Tanzania and Somalia (WHO, 2007a). Another large epidemic hit the Sudan in 2007, in the River Nile Valley around Khartoum (WHO, 2007b). The last outbreak occurred in Madagascar in 2008.

As an opinion of the scientific panel on animal health and welfare was recently adopted on “the risk of Rift Valley fever incursion and its persistence within the Community by the EFSA, we simply reported tables on arthropods naturally infected by RVFV and on vector competence (table 1 and table 2).

**Table 1.** Arthropods naturally infected by RVFV (EFSA, The risk of Rift Valley fever incursion and its persistence within the Community, in *EFSA Journal*. 2005, EFSA. p. 1-128)

Genus (Subgenus)	Species	Locality (year)	Reference
<i>Aedes (Aedimorphus)</i>	<i>cumminsii</i>	Kenya (1981-84)	(Linthicum <i>et al.</i> , 1985)
		Burkina Faso (1983)	(Saluzzo <i>et al.</i> , 1984)
	<i>dalzieli</i>	Senegal (1974, 1983)	(Fontenille <i>et al.</i> , 1998)
	<i>dentatus</i>	Zimbabwe (1969)	(McIntosh, 1972)
	<i>durbanensis</i>	Kenya (1937)	(Mulligan, 1937)
	<i>ochraceus</i>	Senegal (1993)	(Fontenille <i>et al.</i> , 1995)
	<i>tarsalis</i>	Uganda (1944)	(Smithburn <i>et al.</i> , 1948)
	<i>vexans arabiensis</i>	Senegal (1993)	(Fontenille <i>et al.</i> , 1995)
		Saudi Arabia (2000)	(Jupp <i>et al.</i> , 2002)
<i>Aedes</i>	<i>circumluteolus</i>	Uganda (1955)	(Weinbren <i>et al.</i> , 1957)

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(Neomelaniconion)		South Africa (1955, 1981)	(Kokernot <i>et al.</i> , 1957; Jupp <i>et al.</i> , 1983)
	<i>mcintoshii</i>	Zimbabwe (1969)	(McIntosh, 1972)
		South Africa (1974-75)	(McIntosh <i>et al.</i> , 1980)
		Kenya (1981-84)	(Linthicum <i>et al.</i> , 1985)
	<i>palpalis</i>	Central African Republic (1969)	(Digoutte <i>et al.</i> , 1974)
Genus (Subgenus)	Species	Locality (year)	Reference
<i>Aedes</i> ( <i>Ochlerotatus</i> )	<i>caballus</i>	South Africa (1953)	(Gear <i>et al.</i> , 1955)
	<i>juppi</i>	South Africa (1974-75)	(McIntosh <i>et al.</i> , 1980)
<i>Aedes</i> ( <i>Stegomyia</i> )	<i>africanus</i>	Uganda (1956)	(Weinbren <i>et al.</i> , 1957)
	<i>demeilloni/dendrop hilus</i>	Uganda (1944)	(Smithburn <i>et al.</i> , 1948)
<i>Aedes</i> ( <i>Diceromyia</i> )	<i>furcifer</i> group <sup>1</sup>	Burkina Faso (1983)	(Saluzzo <i>et al.</i> , 1984)
<i>Anopheles</i> ( <i>Anopheles</i> )	<i>coustani</i>	Zimbabwe (1969)	(McIntosh, 1972)
		Madagascar (1979)	(Clerc <i>et al.</i> , 1982)
	<i>fuscicolor</i>	Madagascar (1979)	(Clerc <i>et al.</i> , 1982)
<i>Anopheles</i> ( <i>Cellia</i> )	<i>christyi</i>	Kenya (1981-84)	(Linthicum <i>et al.</i> , 1985)
	<i>cinereus</i>	South Africa (1974-75)	(McIntosh <i>et al.</i> , 1980)
	<i>pauliani</i>	Madagascar (1979)	(Clerc <i>et al.</i> , 1982)
	<i>pharoensis</i>	Kenya (1981-84)	(Linthicum <i>et al.</i> , 1985)
	<i>squamosus</i>	Madagascar (1979)	(Clerc <i>et al.</i> , 1982)
	<i>spp.</i> <sup>2</sup>	Madagascar (1979)	(Clerc <i>et al.</i> , 1982)
<i>Culex</i> ( <i>Culex</i> )	<i>antennatus</i>	Nigeria (1967-70)	(Lee, 1979)
		Kenya (1981-84)	(Linthicum <i>et al.</i> , 1985)
	<i>neavei</i>	South Africa (1981)	(McIntosh <i>et al.</i> , 1983)
	<i>pipiens</i>	Egypt (1977, 1978)	(Hoogstraal <i>et al.</i> , 1979; Meegan <i>et al.</i> , 1980)
	<i>poicilipes</i>	Senegal (1998)	(Diallo <i>et al.</i> , 2000)
	<i>theileri</i>	South Africa (1970)	(McIntosh, 1972)
		Zimbabwe (1969)	(McIntosh, 1972)
	<i>tritaeniorhynchus</i>	Saudi Arabia (2000)	(Jupp <i>et al.</i> , 2002)
	<i>vansomereni</i>	Kenya (1981-84)	(Linthicum <i>et al.</i> , 1985)
		South Africa (1981)	(McIntosh <i>et al.</i> , 1983)
	<i>zombaensis</i>	Kenya (1981-84, 1989)	(Linthicum <i>et al.</i> , 1985; Logan <i>et al.</i> , 1991)
<i>Culex</i> ( <i>Eumelanomyia</i> )	<i>rubinotus</i>	Kenya (1981-84)	(Linthicum <i>et al.</i> , 1985)
<i>Eretmapodites</i>	<i>chrysogaster</i>	Uganda (1944)	(Smithburn <i>et al.</i> , 1948)
	<i>quinquevittatus</i>	South Africa (1971)	(McIntosh, 1972)
		Kenya (1981-84)	(Linthicum <i>et al.</i> , 1985)
<i>Coquillettidia</i>	<i>fuscopennata</i>	Uganda (1959)	(Williams <i>et al.</i> , 1960)

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( <i>Coquillettidia</i> )	<i>grandidieri</i>	Madagascar (1979)	(Clerc <i>et al.</i> , 1982)
<i>Mansonia</i> ( <i>Mansoniodes</i> )	<i>africana</i>	Uganda (1959, 1968)	(Williams <i>et al.</i> , 1960; Henderson <i>et al.</i> , 1972)
		Central African Republic (1969)	(Digoutte <i>et al.</i> , 1974)
		Kenya (1989)	(Logan <i>et al.</i> , 1991)
		Uganda (1959)	(Williams <i>et al.</i> , 1960)
	<i>uniformis</i>	Madagascar (1979)	(Clerc <i>et al.</i> , 1982)
Other Diptera	<i>Culicoides spp.</i>	Nigeria (1967)	(Lee, 1979)

<sup>1</sup> Representing a species complex consisting of 3 possible species, *Ae. furcifer*, *Ae. cordellieri* and *Ae. taylora*.

<sup>2</sup> *Culex* spp. may include *annulioris*, *antennatus*, *simpsoni* and *vansomerani*.



**Table 2.** Arthropods for which competence has been demonstrated in laboratory (EFSA, The risk of Rift Valley fever incursion and its persistence within the Community, in *EFSA Journal*. 2005, 1-128)

Genus (Subgenus)	Species	Mode of transmission	Reference
<i>Aedes (Aedimorphus)</i>	<i>fowleri</i>	biological	(Turell <i>et al.</i> , 1988b)
<i>Aedes (Finlaya)</i>	<i>notoscriptus</i>	biological	(Turell and Kay, 1998)
	<i>caballus</i>	biological	(Gear <i>et al.</i> , 1955)
	<i>canadensis</i>	biological	(Gargan <i>et al.</i> , 1988)
	<i>cantator</i>	biological	(Gargan <i>et al.</i> , 1988)
	<i>caspius</i>	biological	(Gad <i>et al.</i> , 1987; Turell <i>et al.</i> , 1996)
	<i>excrucians</i>	biological	(Gargan <i>et al.</i> , 1988)
	<i>juppi</i>	biological	(McIntosh <i>et al.</i> , 1980)
	<i>sollicitans</i>	biological	(Gargan <i>et al.</i> , 1988)
	<i>taeniorhynchus</i>	biological, mechanical	(Hoch <i>et al.</i> , 1985; Gargan <i>et al.</i> , 1988)
<i>Aedes (Neomelaniconion)</i>	<i>circumluteolus</i>	biological	(McIntosh <i>et al.</i> , 1983)
	<i>macintoshi</i>	biological	(McIntosh <i>et al.</i> , 1980)
<i>Aedes (Ochlerotatus)</i>	<i>vigilax</i>	biological	(Turell and Kay, 1998)
<i>Aedes (Protomacleaya)</i>	<i>triseriatus</i>	biological	(Gargan <i>et al.</i> , 1988)
<i>Aedes (Stegomyia)</i>	<i>aegypti</i>	biological, mechanical	(McIntosh <i>et al.</i> , 1980; Hoch <i>et al.</i> , 1985)
	<i>aegypti formosus</i>	mechanical	(Gillet and Mims, 1956)
	<i>albopictus</i>	biological	(Turell <i>et al.</i> , 1988a)
<i>Anopheles (Cellia)</i>	<i>multicolor</i>	biological	(Gad <i>et al.</i> , 1987)
	<i>pharoensis</i>	biological	(Gad <i>et al.</i> , 1987)
<i>Coquillettidia (Coquillettidia)</i>	<i>versicolor</i>	biological	(Daubney and Hudson, 1933)
<i>Culex (Culex)</i>	<i>annulirostris</i>	biological	(Turell and Kay, 1998)
	<i>antennatus</i>	biological	(Gad <i>et al.</i> , 1987; Turell <i>et al.</i> , 1996)
	<i>neavei</i>	biological	(McIntosh <i>et al.</i> , 1973)
	<i>perexiguus</i>	biological	(Turell <i>et al.</i> , 1996)
	<i>pipiens</i>	biological, mechanical	(Hoch <i>et al.</i> , 1985; Turell <i>et al.</i> , 1996)
	<i>poicilipes</i>	biological	(Jupp and Cornel, 1988)
	<i>quinquefasciatus</i>	biological	(McIntosh <i>et al.</i> , 1980)
	<i>salinarius</i>	biological	(Gargan <i>et al.</i> , 1988)
	<i>tarsalis</i>	biological	(Gargan <i>et al.</i> , 1988)
	<i>theileri</i>	biological	(McIntosh <i>et al.</i> , 1973; McIntosh <i>et al.</i> , 1980)
	<i>univittatus</i>	biological	(McIntosh <i>et al.</i> , 1980)
	<i>zombaensis</i>	biological	(McIntosh <i>et al.</i> , 1983)
<i>Culex (Neoculex)</i>	<i>territans</i>	biological	(Gargan <i>et al.</i> , 1988)
<i>Eretmapodites</i>	<i>chrysogaster</i>	biological	(Smithburn <i>et al.</i> , 1949b)

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	<i>quinquevittatus</i>	biological	(McIntosh <i>et al.</i> , 1980)
Other Diptera	<i>Stomoxys calcitrans</i>	mechanical	(Hoch <i>et al.</i> , 1985)
	<i>Glossina morsitans</i>	mechanical	(Hoch <i>et al.</i> , 1985)
	<i>Lutzomyia longipalpis</i>	mechanical	(Hoch <i>et al.</i> , 1985)
	<i>Phlebotomus dubosqi</i>	mechanical	(Dohm <i>et al.</i> , 2000)
	<i>Culicoides variipennis</i>	mechanical	(Hoch <i>et al.</i> , 1985)

Summary as provided by the authors:

- RVFV was isolated from *Aedes vexans*, but not from the subspecies present in Europe. Nevertheless, this latter *Aedes vexans vexans* could be considered a suspected RVFV vector, as *Aedes caspius* and species from the complex *Univittatus* which seems able to support infection with RVFV. According with virus isolation and competence studies, *Culex pipiens* and *Culex theileri* have to be regarded as potential RVFV vectors in Europe.
- Mosquitoes such as *Ae. vexans* could play a role if the virus is introduced. Several *Aedes* species, which breed in wetlands, could transmit the virus. *Culex pipiens*, an ubiquitous vector, is abundant and may amplify the virus in the biological cycle. It is likely that at least one of them would be competent and could initiate a cycle in case of introduction of the virus.

## 1.2. AMERICAN EQUINE ENCEPHALITIS

Eastern equine encephalitis (EEE), Western equine encephalitis (WEE), and Venezuelan equine encephalitis (VEE), limited to the American continent, are caused by *Alphavirus (Togaviridae)*.

### 1.2.1. Eastern equine encephalitis

Eastern equine encephalitis (EEE) virus is recognized to be transmitted between passerine birds by mosquitoes, and rarely to equines or humans (Calisher, 1994). EEE viruses have been isolated in western hemisphere, from Argentina to Canada, and in North America, mainly in the eastern side.

EEE viruses belong to a complex comprising of two varieties of the same subtype. One of them is antigenically relatively homogeneous, regrouping North American and Caribbean isolates, and the other, antigenically less uniform, regrouping South and Central American isolates (Calisher, 1994).

EEE virus infections in equines have been recognized in Cuba for more than 50 years. In Caribbean islands, EEE outbreaks occurred periodically, however, associated human illnesses are rare. EEE virus isolates have been obtained from various sources throughout much of South America, but South American varieties of EEE virus appear to be less virulent for, or less likely to infect, humans (Calisher, 1994). Most of the human cases occurred in the USA, in and around freshwater hardwood swamps in the Atlantic and Gulf Coast states and the Great Lakes region, where approximately 220 cases were confirmed from 1964 to 2004, with an average of 5 cases per year (range from 0 to 15 cases).

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Attempts of EEE virus isolation from field-collected individuals were mainly carried out in the USA (table 3).



**Table 3.** Recent isolations of EEE viruses from mosquitoes

Year	Country	Species	Reference
1997-1998	USA	<i>Culiseta (Climacura) melanura</i> ; MIR <sup>1</sup> = 3.8	(Ortiz <i>et al.</i> , 2003)
1996	USA	<i>Culiseta (Clim.) melanura</i> ; MIR = 8.2 (19 isolations)	(Andreadis <i>et al.</i> , 1998)
1989	USA <sup>2</sup>	<i>Culiseta (Clim.) melanura</i> ; MIR = 11.1 (5 isolations)	(Pagac <i>et al.</i> , 1992)
1985	USA <sup>2</sup>	<i>Culiseta (Clim.) melanura</i> ; MIR = 0.4 (5 isolations)	(Scott <i>et al.</i> , 1987)
1983	USA	<i>Culiseta (Clim.) melanura</i> ; MIR = 4.4 (13 isolations)	(Howard <i>et al.</i> , 1988)
1978-1980	USA	<i>Culiseta (Clim.) melanura</i> ; MIR = 0.1 (3 isolations)	(Howard <i>et al.</i> , 1988)
1972-1974	USA	<i>Culiseta (Clim.) melanura</i> ; MIR max = 10.5 (5 isolations)	(Morris <i>et al.</i> , 1975)
1964-1967	USA	<i>Culiseta (Clim.) melanura</i> ; MIR = 0.5 (23 isolations)	(Bigler <i>et al.</i> , 1976)
1996	USA	<i>Culiseta (Culicella) morsitans</i> ; MIR = 83.3 (3 isolations)	(Andreadis <i>et al.</i> , 1998)
1983	USA	<i>Culiseta (Clim.) morsitans</i> ; MIR = 1.2 (7 isolations)	(Howard <i>et al.</i> , 1988)
1971	USA	<i>Culiseta (Clim.) morsitans</i> ; MIR = 5.3 (3 isolations)	(Morris <i>et al.</i> , 1973)
1996-2001	Peru	<i>Culex (Melanoconion) pedroi</i> (34 isolations)	(Turell <i>et al.</i> , 2005b)
1996-2001		<i>Culex (Mel.) gnomatos</i> (1 isolation)	
1981		<i>Culex (Mel.) panocossa</i> ; MIR = 0.4 (19 isolations)	(Walder <i>et al.</i> , 1984)
		<i>Culex (Mel.) dunni</i> ; MIR = 0.3 (2 isolations)	
1973	Panama	<i>Culex (Mel.) taeniopus</i> ; (1 isolation)	(Dietz <i>et al.</i> , 1980)
1996	USA	<i>Culex (Culex) pipiens</i> ; MIR = 4.2 (8 isolations)	(Andreadis <i>et al.</i> , 1998)
1972-1974	USA	<i>Culex (Culex) restuans</i> (1 isolation)	(Morris <i>et al.</i> , 1975)
1971	USA	<i>Culex (Culex) restuans</i> (1 isolation)	(Morris <i>et al.</i> , 1973)
1964-1967	USA	<i>Culex (Culex) nigripalpus</i> ; MIR < 0.05 (8 isolations)	(Bigler <i>et al.</i> , 1976)
1996-2001	Peru	<i>Aedes (Ochlerotatus) fulvus</i> (1 isolation)	(Turell <i>et al.</i> , 2005b)
1997-1998	USA	<i>Aedes (Ochlerotatus) taeniorhynchus</i> ; MIR = 0.6	(Ortiz <i>et al.</i> , 2003)
1996	USA	<i>Aedes (Ochlerotatus) cantator</i> (1 isolation)	(Andreadis <i>et al.</i> , 1998)
1996	USA	<i>Aedes (Ochlerotatus) sollicitans</i> (2 isolations)	
1982	USA	<i>Aedes (Ochlerotatus) sollicitans</i> ; MIR = 0.1 (3 isolations)	(Crans <i>et al.</i> , 1986)
1996	USA	<i>Aedes (Ochlerotatus) trivittatus</i> (2 isolations)	(Andreadis <i>et al.</i> , 1998)
1983	USA	<i>Aedes (Ochlerotatus) canadensis</i> ; MIR = 0.4 (3 isolations)	(Howard <i>et al.</i> , 1988)
1972-1974	USA	<i>Aedes (Ochlerotatus) canadensis</i> (2 isolations)	(Morris <i>et al.</i> , 1975)
1971	USA	<i>Aedes (Ochlerotatus) stimulans</i> (1 isolation)	(Morris <i>et al.</i> , 1973)
1964-1967	USA	<i>Aedes (Ochlerotatus) atlanticus/tormentor</i> ; MIR = 0.2 (5 isolations)	(Bigler <i>et al.</i> , 1976)
1996	USA	<i>Aedes (Aedimorphus) vexans</i> (1 isolation)	(Andreadis <i>et al.</i> , 1998)
1953	USA	<i>Aedes (Aedimorphus) vexans</i> (1 isolation)	(Wallis <i>et al.</i> , 1960)
1991	USA	<i>Aedes (Stegmyia) albopictus</i> ; MIR max = 1.4 (14 isolations)	(Mitchell <i>et al.</i> , 1992)
1996	USA	<i>Coquillettidia (Coquillettidia) perturbans</i> (1 isolation)	(Andreadis <i>et al.</i> , 1998)

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1991	USA	<i>Coq. (Coq.) perturbans</i> (1 isolation)	(Nasci <i>et al.</i> , 1993)
1964-1967	USA	<i>Coq. (Coq.) perturbans</i> (1 isolation)	(Bigler <i>et al.</i> , 1976)
1996-2001	Peru	<i>Psorophora (Janthinosoma) albigenu</i> (1 isolation)	(Turell <i>et al.</i> , 2005b)

<sup>1</sup> Minimum infection rate per 1,000

<sup>2</sup> Only this species was tested for EEE isolation assays

Relatively few species were recently tested for their ability to be infected with and to transmit EEE virus, exclusively in the USA (table 4).

**Table 4.** Vector competence of mosquitoes for EEE viruses

Species	Mosquito origin	Susceptibility to infection and dissemination <sup>1</sup>	Ability to transmit <sup>2</sup>	Reference
<i>Culiseta (Climacura) melanura</i>	USA	Very high	Very high	(Vaidyana than <i>et al.</i> , 1997)
<i>Culex (Culex) salinarius</i>	USA	Low	Low	(Vaidyana than <i>et al.</i> , 1997)
<i>Aedes (Stegomyia) albopictus</i> <sup>3</sup>	USA	Very high	Moderate	(Turell <i>et al.</i> , 1994)
<i>Aedes (Stegomyia) albopictus</i> <sup>4</sup>	USA	High	Moderate to high	(Scott <i>et al.</i> , 1990)
<i>Aedes (Ochlerotatus) canadensis</i>	USA	Very high	Low	(Vaidyana than <i>et al.</i> , 1997)
<i>Ae. (Ochl.) taeniorhynchus</i> <sup>3</sup>	USA	Moderate	Not competent	(Turell <i>et al.</i> , 1994)
<i>Ae. (Aedimorphus) vexans</i>	USA	Moderate	Not competent	(Vaidyana than <i>et al.</i> , 1997)
<i>Anopheles (Anopheles) punctipennis</i>	USA	Very high	Not competent	(Vaidyana than <i>et al.</i> , 1997)
<i>An. (An.) quadrimaculatus</i>	USA	Moderate	Low	(Vaidyana than <i>et al.</i> , 1997)

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<i>Coquillettidia (Coquillettidia) perturbans</i>	USA	Moderate	Very low	(Vaidyanathan <i>et al.</i> , 1997)
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<sup>1</sup> Classification based on disseminated infection rate: very high > 80%, high 50 to 80%, moderate 20 to 50%, low 5 to 20%, very low < 5%

<sup>2</sup> Classification based on the transmission rate of females with a disseminated infection: very high > 80%, high 50 to 80%, moderate 20 to 50%, low 5 to 20%, very low < 5%

<sup>3</sup> When fed on an older chick with a lower viremia ( $10^{6.1}$  PFU per ml of blood)

<sup>4</sup> Experiments using colonized mosquitoes

Summary as provided by the authors:

- Mosquitoes such as *Ae. vexans* could play a role if the virus is introduced. Several *Aedes* species, which breed in wetlands, could transmit the virus. *Culex pipiens*, an ubiquitous vector, is abundant and may amplify the virus in the biological cycle.
- Based on information from virus isolations, vector competence and mosquito feeding behaviour, it can be stated that the Nearctic species *Culiseta melanura* is the natural enzootic vector<sup>2</sup> of EEE virus in North America. *Culiseta morsitans*, present in northern Europe, should be considered a suspected vector of EEE virus. Due to ornithophilic preferences of *Culiseta melanura*, *Aedes* and *Coquillettidia* species are considered to be epizootic vectors, even if virus isolations from these species are sporadic and they show low ability to transmit the virus in laboratory. From all *Aedes* and *Coquillettidia* species potentially involved in EEE virus transmission (virus isolation), only *Aedes vexans* is present in Europe, but this species has not been shown to be competent for EEE virus (Vaidyanathan *et al.*, 1997). *Aedes albopictus* has been found naturally infected with EEE virus and proven as an experimental competent vector, even if this species has not been implicated in EEE virus transmission. This species, present in some parts of Europe, should be considered a potential vector of EEE virus.

### 1.2.2. Western equine encephalitis

Western equine encephalitis (WEE) virus was first isolated in 1908 during epizootic of equine encephalitis in Argentina (Calisher, 1994). In the 1930s, this virus was recognized to be maintained in bird populations by mosquitoes and leading encephalitis in humans and equines (Calisher, 1994). WEE virus has been found only in Western Hemisphere, from Argentina to Canada, where it can lead equine epizootics throughout its range, whereas human disease in South America occurs less

<sup>2</sup> Enzootic vectors transmit the virus in bird populations, and epizootic vectors (or “bridge” vectors) from birds to mammals (mainly humans and equines for these viruses).



frequently and with less severity than in North America. In North America, isolations of WEE virus attest to the wide distribution of this virus in western North America (Calisher, 1994).

In North America, major epizootics and large epidemics occurred in the 1930s, the 1940s and the beginning of the 1950s. From 1955 to 1984, an annual average of 34 (range, 0 to 172) confirmed human cases of WEE occurred in the USA. But, since 1988, only few human cases were reported (Calisher, 1994).

WEE virus was first isolated from mosquitoes in 1941, from field-collected *Culex tarsalis* females in the USA (Hammon *et al.*, 1941). Isolation assays were mainly carried out in this country (table 5).





**Table 5.** Recent isolations of WEE viruses from mosquitoes

Year	Country	Species	Reference
1994-1995	USA <sup>1</sup>	<i>Culex (Culex) tarsalis</i> ; MIR <sup>2</sup> max = 3.8	(Janousek and Kramer, 1998)
1991-1994	USA	MIR max = 3.9	(Reisen <i>et al.</i> , 1997b)
1983-1987	USA	113 isolations, MIR max = 2.0	(Reisen <i>et al.</i> , 1990)
1987-1988	USA	6 isolations, MIR max = 3.3	(Jakob <i>et al.</i> , 1989)
1985	Mexico	6 isolations, MIR max = 2.4	(Clark <i>et al.</i> , 1986)
1962-1973	USA	567 isolations, MIR max = 2.0	(Olson <i>et al.</i> , 1979)
1972	USA	111 isolations, MIR max = 6.6	(Hayes <i>et al.</i> , 1976)
1972	Mexico	1 isolation, MIR = 3.5	(Sudia <i>et al.</i> , 1975a)
1962	Canada	3 isolations	(Spalatin <i>et al.</i> , 1963)
1941	USA	1 isolation	(Hammon <i>et al.</i> , 1941)
1987-1988	USA	<i>Culex (Culex) erythrothorax</i> ; 4 isolations, MIR max = 0.5	(Jakob <i>et al.</i> , 1989)
1972	USA	<i>Culex peus</i> ; 7 isolations, MIR max = 8.5	(Hayes <i>et al.</i> , 1976)
1996-2001	Peru	<i>Aedes (Ochlerotatus) hastatus</i> ; 1 isolation	(Turell <i>et al.</i> , 2005b)
1991-1992	USA	<i>Aedes (Ochl.) dorsalis</i> ; isolations from larvae	(Fulhorst <i>et al.</i> , 1994)
1985	Mexico	3 isolations, MIR max = 0.5	(Clark <i>et al.</i> , 1986)
1972	USA	5 isolations, MIR max = 3.0	(Hayes <i>et al.</i> , 1976)
1962	Canada	1 isolation	(Spalatin <i>et al.</i> , 1963)
1983-1987	USA	<i>Aedes (Ochl.) melanimon</i> ; 13 isolations, MIR max = 0.2	(Reisen <i>et al.</i> , 1990)
1985	Mexico	<i>Aedes (Ochl.) campestris</i> ; 9 isolations, MIR = 0.9	(Clark <i>et al.</i> , 1986)
1982	Argentina	<i>Aedes (Ochl.) albifasciatus</i> ; 1 isolation, MIR < 0.1	(Mitchell <i>et al.</i> , 1987)
1962	Canada	<i>Aedes (Ochl.) flavescens</i> ; 1 isolation	(Spalatin <i>et al.</i> , 1963)
1987-1988	USA	<i>Aedes (Aedimorphus) vexans</i> ; 1 isolation, MIR max = 0.3	(Jakob <i>et al.</i> , 1989)
1982	USA	<i>Aedes (Aedimorphus) vexans</i> ; 7 isolations, MIR max = 0.3	(Hayes <i>et al.</i> , 1976)
1983-1987	USA	<i>Culiseta (Culiseta) inornata</i> ; 1 isolation, MIR max = 0.1	(Reisen <i>et al.</i> , 1990)
1962	Canada	1 isolation	(Spalatin <i>et al.</i> , 1963)
1966	USA	<i>Culiseta (Climacura) melanura</i> ; 24 isolations	(Muul <i>et al.</i> , 1975)
1982	Argentina	<i>Psorophora (Psorophora) pallescens</i> ; 1 isolation, MIR = 4.6	(Mitchell <i>et al.</i> , 1987)
1972	USA	<i>Psorophora (Grabhamia) columbia</i> ; 5 isolations, MIR = 4.5	(Hayes <i>et al.</i> , 1976)
1972	USA	<i>Psorophora (Grabhamia) signipennis</i> ; 1 isolation, MIR = 0.5	(Hayes <i>et al.</i> , 1976)
1982	Argentina	<i>Anopheles (Nyssorhynchus) albitarsis</i> ; 1 isolation, MIR = 1.1	(Mitchell <i>et al.</i> , 1987)

<sup>1</sup> Only *Culex* species were tested for WEE isolation assays

<sup>2</sup> Minimum infection rate per 1,000 mosquitoes

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Vector competence of *Cx. tarsalis* has been widely studied, compared to other species, thus we reported only selected papers for *Cx. tarsalis* (table 6).



**Table 6.** Vector competence of mosquitoes for WEE viruses

Species	Mosquito origin	Susceptibility to infection and dissemination <sup>1</sup>	Ability to transmit <sup>2</sup>	Reference
<i>Culex (Culex) tarsalis</i> <sup>3</sup>	USA	High	Moderate	(Reisen <i>et al.</i> , 1990)
	USA	High	Low to moderate	(Reisen <i>et al.</i> , 1996)
	USA	Moderate to very high	Low to high	(Reisen <i>et al.</i> , 1997a)
<i>Culex (Culex) pipiens</i>	Argentina	Very low	Not tested	(Aviles <i>et al.</i> , 1990)
<i>Culex (Cx.) quinquefasciatus</i>		Not efficient	Not tested	
<i>Aedes (Ochlerotatus) dorsalis</i> <sup>3</sup>	USA	High	Low to high	(Kramer <i>et al.</i> , 1998)
<i>Aedes (Ochl.) albifasciatus</i>	Argentina	High to very high	Not tested	(Aviles <i>et al.</i> , 1990)
<i>Aedes (Ochl.) trivittatus</i>	USA	Low	Not tested	(Green <i>et al.</i> , 1980)
<i>Culiseta (Climacura) melanura</i> <sup>4</sup>	USA	High to very high	High to very high	(Hayes, 1979)

<sup>1</sup> Classification based on disseminated infection rate: very high > 80%, high 50 to 80%, moderate 20 to 50%, low 5 to 20%, very low < 5%

<sup>2</sup> Classification based on the transmission rate of females with a disseminated infection: very high > 80%, high 50 to 80%, moderate 20 to 50%, low 5 to 20%, very low < 5%

<sup>3</sup> Artificial feeding methods

<sup>4</sup> Experiments using colonized mosquitoes

Summary as provided by the authors:

- Based on information from virus isolations, vector competence and mosquito feeding behaviour, it can be stated that *Culex tarsalis* is the natural vectors of WEE virus. Its density is correlated with the disease incidence (Olson *et al.*, 1979), and its western distribution in North America is superimposed on the classical disease distribution. In eastern parts of the USA, where *Cx. tarsalis* is absent, *Culiseta melanura* is considered the maintenance vector. Both species are restricted to North America. In South America, WEE virus vectors remain unknown. *Aedes* species are suspected to play a role in virus persistence through vertical transmission (Fulhorst *et al.*, 1994).
- Aedes dorsalis* is the only species tested competent for WEE virus and present in Europe, but according with its mammophilic behaviour and the low level of infection in the field compared to *Cx. tarsalis*, this species probably do not play an important role in transmission of WEE

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virus. *Aedes vexans*, which is present in Europe, has been found sporadically infected with WEE virus, but it has never been implicated in WEE virus transmission and its vector competence has never been tested.

### 1.2.3. Venezuelan equine encephalitis

Venezuelan equine encephalitis (VEE) was first recognised as a disease of horses, donkeys and mules in the mid-1930s, and as a cause of human disease (mainly febrile illness, sometimes neurological manifestations, and occasional mortality) in the late 1950s (Weaver and Barrett, 2004).

Rodents were thought to be reservoir hosts of the VEE viruses. These latter belong to a complex including epizootic strains (serotypes IAB and IC) and enzootic strains (several species separated into six subtypes). Enzootic strains generated little or no viraemia in experimentally infected horses, and were thought incapable of causing epidemics. These strains were present in sylvatic cycles in several tropical and subtropical regions of South and Central America as well as in Mexico and Florida (Weaver and Barrett, 2004). On the contrary, horses developed a high titre viraemia following the infection with epizootic strains, and thus serve as efficient amplifying hosts.

From 1920 to 1970, periodic but unpredictable outbreaks of VEE (some involving hundreds of thousands of equine and human cases) occurred in the northern part of South America. One of these epizootics started in 1969 in El Salvador and Guatemala to reach the South of the USA in 1971. After a period of silence from 1973 to 1992, new equine and human outbreaks occurred in Venezuela 1992/93 and in Venezuela and Colombia 1995, associated during these years with small equine epizootics in Mexico (Weaver and Barrett, 2004).

In 1971, 41 species belonging to 11 genera of mosquitoes were reported naturally infected with endemic viruses of the VE complex. Of these, 20 belong to the genus *Culex* and 13 to the subgenus *Melanoconion* (Galindo, 1972). Of a total of 406 isolations reported from naturally infected mosquitoes, 286 (or 70%) were obtained from *Culex (Melanoconion)* females (Galindo, 1972). Table 7 lists VEE isolations from mosquitoes published since this date.

**Table 7.** Recent isolations of VEE viruses from mosquitoes

Year	Country	Strain	Species	Reference
2003	Peru	Enzootic	<i>Culex (Melanoconion) gnomatos</i>	(Yanoviak <i>et al.</i> , 2005)
1996-2001	Peru	Enzootic	<i>Culex (Mel.) gnomatos</i>	(Turell <i>et al.</i> , 2005b)
			<i>Culex (Mel.) vomerifer/gnomatos</i>	
			<i>Culex (Mel.) ocosa</i>	
			<i>Culex (Mel.) pedroi</i>	
			<i>Culex (Mel.) spissipes</i>	

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Year	Country	Strain	Species	Reference
1982	Argentina	-	<i>Culex (Mel.) delpontei</i> (MIR <sup>1</sup> = 39.0)	(Mitchell <i>et al.</i> , 1987)
			<i>Psorophora pallescens</i> (MIR = 4.6)	
			<i>Anopheles albitarsis</i> (MIR = 1.1)	
			<i>Aedes albifasciatus</i> (MIR < 0.1)	
1982	Argentina	Enzootic	<i>Culex (Mel.) ocosa</i> group (MIR = 1.6)	(Mitchell <i>et al.</i> , 1985)
			<i>Culex (Mel.) delpontei</i> (3 isolates, MIR = 2.5; 1.4 and 1.8)	
			<i>Aedes scapularis</i> (MIR = 100.0; 1 pool)	
1977-1980	Guatemala	-	<i>Culex (Mel.) taeniopus</i>	(Cupp <i>et al.</i> , 1986)
1972	Mexico	Epidemic	<i>Anopheles p. pseudopunctipennis</i>	(Sudia <i>et al.</i> , 1975a)

1971	Mexico	Epidemic	<i>Psophorora confinnis</i> (MIR = 8.2) <sup>2</sup>	(Sudia <i>et al.</i> , 1975b)
			<i>Psophorora discolour</i> (MIR = 12.5)	
			<i>Aedes sollicitans</i> (MIR = 4.9)	
			<i>Aedes thelcter</i> (MIR = 20.8)	
			<i>Psophorora ciliate</i> (MIR = 17.9)	
			<i>Psophorora cyaneus</i> (MIR = 10.0)	
			<i>Culex tarsalis</i> (MIR = 12.5)	
1971	Texas	Epidemic	<i>Aedes sollicitans</i> (MIR = 3.8) <sup>2</sup>	(Sudia <i>et al.</i> , 1975b)
			<i>Deinocerites pseudes</i> (MIR = 0.4)	
			<i>Psophorora confinnis</i> (MIR = 0.7)	
			<i>Psophorora discolor</i> (MIR = 5.6)	
			<i>Aedes thelcter</i> (MIR = 2.4)	
			<i>Anopheles crucians</i> (MIR = 0.2)	
			<i>Aedes taeniorhynchus</i> (MIR = 3.6)	
1963-1964	Mexico	-	<i>Culex (Culex) iolambdis</i> (MIR = 0.2 to 14.7)	(Scherer <i>et al.</i> , 1971)
			<i>Culex (Cx.) thriambus</i> (MIR = 50.0; 4 pools)	
			<i>Culex (Cx.) coronator</i> (MIR = 50.0; 1 pool)	
			<i>Culex (Mel.) opisthopus</i> (MIR = 12.9 - 20)	
			<i>Deinocerites pseudes</i> (MIR = 0.5)	
			<i>Haemagogus mesodentatus</i> (MIR = 76.9; 13 individuals)	
			<i>Wyeomyia mitchelli</i> (MIR = 8.1)	

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Year	Country	Strain	Species	Reference
			<i>Aedes scapularis</i> (MIR = 35.7; 1 pool)	

<sup>1</sup> Minimum infection rate per 1,000 mosquitoes

<sup>2</sup> Species sorted by abundance. The most abundant species were in Mexico: *P. confinnis* (85% of the collection), *P. discolour* (7%) and *Ae. sollicitans* (4%), and in Texas: *Ae. sollicitans* (40% of the collection), *D. pseudes* (18%), *P. confinnis* (13%), *P. discolor* (4%), *Ae. thelcter* (3%), *An. crucians* (3%) (Sudia *et al.*, 1975b).

Almost all mosquito species tested for the ability to transmit VEE viruses were obviously American species (table 8).





**Table 8.** Vector competence of mosquitoes for VEE viruses

Species	Mosquito origin	Strain	Susceptibility to infection and dissemination <sup>1</sup>	Ability to transmit <sup>2</sup>	Reference
<i>Culex (Melanoconion) gnomatos</i>	Peru	Enzootic	Very high	High	(Turell <i>et al.</i> , 2006)
<i>Cx. (Mel.) gnornatos/vomerifer</i>	Peru	Enzootic/ Epidemic	High	High	(Turell <i>et al.</i> , 2000; Turell <i>et al.</i> , 2006)
<i>Culex (Mel.) taeniopus</i>	Mexico	Enzootic	High	High	(Turell <i>et al.</i> , 2003)
	Guatemala <sup>3</sup>	Enzootic/ Epidemic	Very low to very high	Moderate	(Scherer <i>et al.</i> , 1987)
<i>Cx. (Mel.) spissipes</i>	Peru	Enzootic	High	Moderate (few specimens)	(Turell <i>et al.</i> , 2006)
<i>Cx. (Mel.) vomerifer</i>	Peru	Enzootic	Low	Low	
<i>Cx. (Mel.) pedroi</i>	Peru	Enzootic/ Epidemic	Very low to moderate	Low	(Turell <i>et al.</i> , 2000; Turell <i>et al.</i> , 2006)
<i>Cx. (Mel.) portesi</i>	Peru	Enzootic	Low	Very low	(Turell <i>et al.</i> , 2006)
<i>Cx. (Mel.) theobaldi</i>	Peru	Enzootic	Low	Very low	
<i>Culex (Deinocerites) pseudes</i>	Mexico	Enzootic/ Epidemic	High	High	(Turell <i>et al.</i> , 2003)
<i>Culex (Dein.) cancer</i>	Honduras	Enzootic	Low	Not tested	
<i>Culex (Culex) coronator</i>	Peru	Enzootic/ Epidemic	Very low to low	Not efficient	(Turell <i>et al.</i> , 2000)
<i>Cx. (Culex) declarator</i>	Venezuela	Enzootic	Not efficient	Not efficient	(Turell, 1999)
<i>Cx. (Culex) nigripalpus</i>	USA	Enzootic	Not efficient		
<i>Psorophora (Grabhamia) columbiae</i> sl	Mexico USA	Enzootic/ Epidemic	High to very high	High to very high	(Moncayo <i>et al.</i> , 2008)
<i>Ps. (Gra.) confinnis</i>	USA	Enzootic/ Epidemic	High to very high	High to very high	(Ortiz <i>et al.</i> , 2005)
<i>Ps. (Gra.) cingulata</i>	Peru	Enzootic/ Epidemic	High	Low	(Turell <i>et al.</i> , 2000 ; Turell <i>et al.</i> , 2006)
<i>Ps. (Janthinosoma) albigena</i>	Peru	Enzootic	Moderate to high	Very low to low	(Turell <i>et al.</i> , 2000 ; Turell <i>et al.</i> , 2006)
		Epidemic	Very high	Very low to low	
<i>Ps. (Jan.) ferox</i>	Peru	Enzootic/ Epidemic	Moderate	Not efficient	(Turell <i>et al.</i> , 2000 ; Turell <i>et al.</i> , 2006)

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<i>Ae. (Stegomyia) albopictus</i>	USA	Epidemic	Very high	High	(Smith <i>et al.</i> , 2005)
	Brasil	Enzootic/ Epidemic	Moderate to very high	Moderate to high	(Fernandez <i>et al.</i> , 2003)
	USA <sup>3</sup>	Epidemic	Very high	Moderate	(Turell <i>et al.</i> , 1992)

Species	Mosquito origin	Strain	Susceptibility to infection and dissemination <sup>1</sup>	Ability to transmit <sup>2</sup>	Reference
<i>Aedes (Ochlerotatus) taeniorhynchus</i> <sup>2</sup>	USA	Epidemic	High	High	(Smith <i>et al.</i> , 2005)
	USA	Enzootic	Not efficient		(Coffey and Weaver, 2005)
		Epidemic	Very high	Not tested	
	USA	Enzootic	Moderate	Low to moderate	(Ortiz and Weaver, 2004)
		Epidemic	Moderate to very high	Low to high	
	Mexico Honduras	Epidemic	High to very high	High to very high	(Turell <i>et al.</i> , 2003)
		Enzootic	Low	Very low	
	Venezuela	Epidemic	Very high	Very high	(Turell, 1999)
	USA	Epidemic	Moderate to high	Low	(Turell and Beaman, 1992)
<i>Ae. (Och.) sollicitans</i>	USA	Epidemic	High to very high	Moderate	
<i>Ae. (Och.) fulvus</i>	Peru	Enzootic/ Epidemic	Moderate to very high	Very low to low	(Turell <i>et al.</i> , 2000; Turell <i>et al.</i> , 2006)
<i>Ae. (Och.) serratus</i>	Peru	Enzootic/ Epidemic	Very low to low	Not efficient	
<i>Coquilettidia (Rhynchotaenia) venezuelensis</i>	Peru	Enzootic/ Epidemic	Very low to high	Not tested	(Turell <i>et al.</i> , 2000)
<i>Mansonia (Mansonia) indubitans/titillans</i>	Peru	Enzootic/ Epidemic	Moderate	Low	(Turell <i>et al.</i> , 2000)
<i>Mansonia (Man.) titillans</i>	Venezuela	Epidemic	High	Not tested	(Turell, 1999)

<sup>1</sup> Classification based on disseminated infection rate: very high > 80%, high 50 to 80%, moderate 20 to 50%, low 5 to 20%, very low < 5%

<sup>2</sup> Classification based on the transmission rate of females with a disseminated infection: very high > 80%, high 50 to 80%, moderate 20 to 50%, low 5 to 20%, very low < 5%

<sup>3</sup> Experiments using colonized mosquitoes

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Summary as provided by the authors:

- Based on virus isolation from field-collected individuals, on vector competence and on mosquito feeding behaviour, it can be stated that *Culex (Melanoconion)* species are the natural vectors of enzootic VEE viruses. The epizootic strains are transmitted by other genera mainly *Psorophora (Grabhamia)* species and *Aedes (Ochlerotatus)* species such as *Ae. taeniorhynchus* or *Ae. sollicitans*, and large outbreaks in human or equine populations may be due to the adaptation of epizootic strains to these vector species (Ortiz and Weaver, 2004). The distribution of both subgenus *Melanoconion* and genus *Psorophora* is restricted to the American continent. *Aedes albopictus* is the only species tested competent for VEE viruses and present in Europe, but this species has never been implicated in transmission in the field.

### 1.3. DENGUE, YELLOW FEVER AND CHIKUNGUNYA FEVER

#### 1.3.1. Dengue

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are diseases caused by four dengue virus serotypes called DEN-1, DEN-2, DEN-3 and DEN-4. In tropical and sub-tropical countries, it has been estimated that 2.5 billion people, living mostly in large and small cities are at risk to infection with one or more dengue viruses (Halstead, 2007). About 50 to 100 million individuals are infected every year, and in some years as many as 500,000 people have been admitted to hospital (Halstead (1988) in Halstead (2007)).

Dengue virus infection in humans causes a spectrum of manifestations ranging from unapparent or mild febrile illness to severe and fatal hemorrhagic disease.

Classic dengue fever (DF) is primarily a disease of older children and adults. It is characterized by the sudden onset of fever and a variety of nonspecific signs and symptoms, including frontal headache, retro-orbital pain, body aches, nausea and vomiting, joint pain, weakness and rash. DF is generally self-limited and rarely fatal. The acute phase of illness last for 3 to 7 days but the convalescence phase may be prolonged for weeks. No permanent sequels are known to be associated with this infection.

Dengue hemorrhagic fever (DHF) is primarily a disease of children under the age of 15 years, although it may also occur in adults. It is characterized by sudden onset of fever, which usually lasts for 2 to 7 days, and a variety of nonspecific signs and symptoms. During the acute phase it is difficult to distinguish DHF from DF and other illnesses found in tropical areas. The critical stage in DHF is at the time of defervescence. DHF is characterized by rapid onset of capillary leakage accompanied thrombocytopenia, altered haemostasis, and damage to the liver. Common hemorrhagic manifestations include skin haemorrhage such as petechiae, purpuric lesions and ecchymoses. Epistaxis, bleeding gums, gastrointestinal haemorrhage and haematuria occur less frequently. In the most severe form (designated dengue shock syndrome DSS) the blood vessels can collapse, causing circulatory failure. The case-fatality rate of DHF/DSS is up to 20% if untreated,



but with supportive treatment consisting of fluid and electrolyte management and oxygen, less than 1% of such cases prove to be lethal.

Tools available to prevent dengue infection are very limited. There is no vaccine currently available and prevention is only based on vector control.

The primitive enzootic transmission cycles of dengue viruses involves canopy-dwelling *Aedes* mosquitoes and lower primates in the rain forest of Asia and Africa. Current evidence suggests that these viruses do not regularly move out of forest to urban areas (Rico-Hesse, 1990).

**Table 9.** Vector competence studies and field isolation with dengue viruses

Species	Dengue		Cycle		Habits	Distribution						
			Dom <sup>†</sup>	Sylv		Afr		Asia		Oc	Am	
	Lab studies	Field isolation			Anthr	W	E	ME	SE		SC	N
<i>Ae. aegypti</i>	(Tran Khanh <i>et al.</i> , 1999; Vazeille-Falcoz <i>et al.</i> , 1999; Vazeille <i>et al.</i> , 2001; Vazeille <i>et al.</i> , 2003)	(Rudnick, 1966; Russell <i>et al.</i> , 1969; Fauran <i>et al.</i> , 1984; Diallo <i>et al.</i> , 2003)			df							
<i>Ae. albopictus</i>	(Gratz, 2004)	(Rudnick, 1966; Gratz, 2004)										
<i>Ae. polynesiensis</i>	(Rosen <i>et al.</i> , 1954)											
<i>Ae. vigilax</i>		(Fauran <i>et al.</i> , 1984)										
<i>Ae. furcifer</i>		(Diallo <i>et al.</i> , 2003)										
<i>Ae. taylori</i>		(Diallo <i>et al.</i> , 2003)										
<i>Ae. luteocephalus</i>		(Diallo <i>et al.</i> , 2003)										
<i>Ae. vittatus</i>	(Mavale <i>et al.</i> , 1992)	(Diallo <i>et al.</i> , 2003)										
<i>Ae. niveus</i>		(Rudnick, 1978)										

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<i>Cx. annulirostris</i>		(Fauran <i>et al.</i> , 1984)											
<i>Coq. xanthogaster</i>		(Fauran <i>et al.</i> , 1984)											

<sup>1</sup>Dom: domestic, Sylv.: syvatic, Anthr.: anthropophilic, Afr.: Africa, Oc.: Oceania, Am.: America, Eur.: Europe, W: West, E: East, M-E: Middle-East, S-E: South-East, S-C: South and Central, N: North, df : domestic form.

**Forest enzootic cycle.** In Senegal, DEN-2 virus was isolated on mosquito cell lines from *Ae. furcifer*, *Ae. taylori*, *Ae. luteocephalus*, *Ae. vittatus* and *Ae. aegypti* (sylvatic form), collected in the forest gallery from Kedougou (Diallo *et al.*, 2003). *Aedes taylori* and *Ae. luteocephalus*, because of their scarcity in villages may have a role limited to the forest gallery. However, *Ae. furcifer* may contribute to both sylvatic transmission and virus dissemination from the forest zone to human habitat since this species is the only infected one abundant in the domestic environment. *Aedes aegypti* sylvatic form has probably a limited role in sylvatic amplification cycles of DENV-2. This form has a low human biting rate and tends to be more zoophilic. *Aedes vittatus* was found infected by DENV-2. In one study, *Ae. vittatus* mosquitoes were infected by oral route and by intrathoracic inoculation with dengue (DEN) viruses and tested for the presence of dengue virus antigen in their head squashes and salivary glands by indirect immunofluorescence. The results indicate that this species was susceptible to all four types of DEN viruses and supported the growth of DEN-2 virus (Mavale *et al.*, 1992). This anthropophilic species is largely distributed and is already present in Europe (around the occidental Mediterranean basin).

In Asia, a silent jungle transmission cycle of dengue virus has also been described (Knudsen, 1977). In Malaysia, DENV-4 was isolated from a pool of *Ae. niveus* subgroup (Rudnick, 1978). This represented the first confirmed isolation of dengue virus from a jungle mosquito in nature. Some other species was suspected to be involved in this jungle transmission cycle like *Ae. amesii*, *Armigeres* species and *Coquillettidia* species (Knudsen, 1977). *Aedes albopictus* was also suspected act as a vector in a sylvan cycle of dengue (Scanlon, 1965).

**Rural epidemic cycle.** An epidemic transmission cycle may occur in rural villages, where the human population is small. Introduced viruses quickly infect the majority of susceptible individuals in these areas, and increasing herd immunity causes the virus to disappear from the population. A number of *Aedes* (*Stegomyia*) spp. may act as vectors in these situations, depending on the geographic area, including *Ae. aegypti*, *Ae. albopictus*, *Ae. polynesiensis* and other members of the *Ae. scutellaris* group.

Several Chinese studies cited by (Gratz, 2004) have demonstrated the status of *Ae. albopictus* as a dengue vector in China by field isolations and experimental transmission studies. It has been shown that *Ae. albopictus* has a lower oral receptivity to DENV-2 than that *Ae. aegypti* (Vazeille *et al.*, 2003). However male *Ae. albopictus* can transmit dengue virus sexually during mating and females can transmit it vertically more efficiently than can *Ae. aegypti* females (Vazeille *et al.*, 2003). This species may so contribute to the maintenance of the virus during interepidemic periods. When the only present vector is *Ae. albopictus* it can be responsible for dengue epidemics, as was shown for the outbreaks in the Seychelles and Japan, and the more recent small outbreak in Hawaiï (2001-

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2002). *Ae. albopictus* has recently spread and is now quite worldwide distributed (see review (Gratz, 2004)). Before 1979, *Ae. albopictus* was widely spread in an area from the Pacific to Madagascar and the Seychelles, in the Indian Ocean; in the north it was present in China, Korea and Japan. In warmer areas it was found throughout most of the South-east Asia region and west to Hawaii. However, in 1979 the species was found for the first time in Europe, in Albania. It had probably been imported in tire shipments from China. *Aedes albopictus* was then detected in the United States in 1985. In 1986 it was detected in Brazil and Mexico became the next positive country in 1988. Between 1988 and 1995, the species was found in most of Central America, and on some parts of the Caribbean islands after 1993. From 1993 to 2003, it was detected in most of the South American countries. In the Pacific area it was discovered in Salomon, Australia (1988), Fidji (1988), New Zealand (1994), and La Reunion (1994). In Africa the species was established in South Africa (1990), Nigeria (1991) and southern Cameroon. In September 1990, an infestation of *Ae. albopictus* was discovered in the city of Genoa in Italy. This mosquito has rapidly spread and was present in 9 regions in 2001. *Aedes albopictus* was found for the first time in France in 1999 and in 2000 in one location in Belgium. It has also been reported in Montenegro, Hungary and Israel. In the South Pacific area, DENV-1 was recovered from *Ae. aegypti*, *Ae. polynesiensis* and *Ae. vigilax*. DENV-4 was obtained from *Ae. aegypti*, *Ae. vigilax*, *Coq. xanthogaster* and *Cx. annulirostris* (Fauran *et al.*, 1984). *Aedes polynesiensis* was shown to be capable of transmitting dengue from monkey to monkey in the laboratory (Rosen *et al.*, 1954). This mosquito species has its distribution limited to the South Pacific Islands. This mosquito colonizes coconut shells, rock holes and crab holes but is now adapted to a wide variety of water-filled man-made containers such as buckets, discarded tyres and drums, wells and water cisterns.

**Urban endemic/epidemic cycle.** The most important transmission cycle from a public health standpoint is the urban endemic/epidemic cycle in large urban centres of the tropics. The viruses are maintained in an *Ae. aegypti* – human – *Ae. aegypti* cycle with periodic epidemics. The oral susceptibility of *Ae. aegypti* for dengue viruses has now largely been studied (see for example (Tran Khanh *et al.*, 1999; Vazeille-Falcoz *et al.*, 1999; Vazeille *et al.*, 2001; Vazeille *et al.*, 2003)). Sometimes *Ae. albopictus* is also involved in these urban epidemics (Rudnick, 1966). However, *Ae. aegypti* is the major vector of DF/DHF in tropical and subtropical areas and dengue cases are now reported from virtually every global locations in which *Ae. aegypti* occurs.

Summary as provided by the authors:

- *Aedes albopictus*, which has been introduced in several European countries since 1975, is a proven vector of dengue. Mediterranean dengue outbreaks were transmitted by *Ae. aegypti* during the 19th and the beginning of the 20th century. This species has been eradicated in Europe during the first half of the 20th century, but remains at risk of re-introduction in Europe. Because *Ae. vittatus* has been naturally found infected by dengue virus and it is able to multiplied the virus after experimental infection it should be considered a potential vector, even if it has never been responsible for dengue epidemics.

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### 1.3.2. Yellow fever

Yellow fever is a viral hemorrhagic fever produced by an arthropod-borne virus of the genus *Flavivirus* and *Flaviviridae* family. It is an infectious non-contagious disease maintained by transmission between primates and mosquitoes.

After the bite of an infected mosquito, the incubation period for Yellow fever is generally 3-6 days. The illness is divided in 3 phases: i) infection that lasts 3-6 days and includes symptoms as fever, headache, chills, malaise, anorexia, nausea vomiting and lumbosacral myalgia, ii) remission and abortive phase, and iii) intoxication with haemorrhages and liver and renal failures preceding the dead. Approximately 85-90% of infections are mild or asymptomatic including the first and second previously commented phases, the resting 10-15% results in severe disease with 50% fatality rate.

The diagnosis of YF is difficult to differentiate from many other diseases at the early stage of the infection and while the disease is ongoing. The definitive diagnosis is performed by serology or by virus isolation

There is no specific treatment for YF infection. A highly effective live attenuated vaccine was developed using the YF virus strain 17D. Vaccination is performed in residents of endemic zones and WHO strongly recommends the vaccination in citizens planning to travel to epidemic or endemic countries, even in the absence of reported cases.

Yellow fever is endemic in many parts of West Africa. Epidemics in South America have been reduced due to intensive vector control campaigns and mass vaccination with satisfactory results. Actions taken to prevent transmission in epidemics are vector control, mass vaccination and surveillance. Epidemiological patterns of YF virus transmission can be divided in three different scenarios: sylvatic or forest cycle, urban cycle and the intermediate cycle that links sylvatic and urban cycles.

In Africa the **sylvatic cycle** is maintained by transmission between non-human primates and *Aedes* mosquitoes of the genus *Stegomyia* and *Diceromyia*, especially *Aedes africanus*. Sylvatic cycle is maintained in tropical forests with viral transmission between non-human primates and fierce mosquitoes. The **intermediary cycle** is found in the humid and semi-humid African savannas with viral transmission between human and non-human primates involving fierce (*Ae. bromeliae*, *Ae. fuscifer*, *Ae. luteocephalus* and *Ae. africanus*) and domestic (*Ae. aegypti*) mosquitoes (Faye *et al.*, 2007). Since the 1940s, *Ae. simpsoni* has been considered an important YF vector in East and West Africa linking sylvatic and domestic cycles. In the Ethiopian region three morphologically distinct forms within *Ae. simpsoni* were identified: *Ae. simpsoni*, *Ae. bromeliae* and *Ae. lilii* (Huang, 1979a) and further studies incriminated *Ae. bromeliae* as the YF vector in East Africa (Huang, 1986). Citations of *Ae. simpsoni* before 1979 must be referred as *Ae. simpsoni* group.





*Phlebotominae* have been incriminated with YF when the virus was isolated from *Phlebotomine* sand flies in Bwamba (Africa) (Smithburn *et al.*, 1949a). No further studies have been performed to follow up this finding that can explain cases of YF immunity in Monkeys in areas where no YF virus has been detected in *Aedes* mosquitos (McCrae and Kirya, 1982).

In South America the sylvatic cycle is maintained in tropical forests with viral transmission between non-human primates and *Haemogogus* and *Sabethes* mosquitoes in epizootic cycle. Indigenous neotropical monkeys have higher mortality rates for YF virus in comparison to the African monkeys, this is thought to be one of the reasons for the special ecology of YF in South America where the virus moves from place to place in epizootic waves rather than being persistently in endemic regions with transmission between monkeys and mosquitoes.



**Table 10.** Vector competence studies and field isolation with yellow fever viruses

Species	Yellow fever		Cycle <sup>1</sup>		Habits	Distribution							
			Dom	Sylv	Anthr	Afr		Asia		Oc	Am		Eur
	Lab studies	Field isolation				W	E	ME	SE		SC	N	
<i>Ae. aegypti</i>	(Cordellier <i>et al.</i> , 1974; Aitken <i>et al.</i> , 1979; Vainio and Cutts, 1998; Johnson <i>et al.</i> , 2002; Jupp and Kemp, 2002; Faye <i>et al.</i> , 2007)	(Cordellier <i>et al.</i> , 1974; Fontenille <i>et al.</i> , 1997)			df								
<i>Ae. albopictus</i>	(Johnson <i>et al.</i> , 2002; Lourenco de Oliveira <i>et al.</i> , 2003; Vazeille <i>et al.</i> , 2008)												
<i>Ae. taylori</i>	(Cordellier <i>et al.</i> , 1974; Vainio and Cutts, 1998)	(Cornet <i>et al.</i> , 1979) furc-tayl. group											
<i>Ae. luteocephalus</i>	(Cordellier <i>et al.</i> , 1974; Vainio and Cutts, 1998)	(Cordellier <i>et al.</i> , 1974; Cornet <i>et al.</i> , 1979; Germain <i>et al.</i> , 1982; Fontenille <i>et al.</i> , 1997)											
<i>Ae. africanus</i>	(Cordellier <i>et al.</i> , 1974; Vainio and Cutts, 1998)	(Cordellier <i>et al.</i> , 1974; Kirya <i>et al.</i> , 1977; Germain <i>et al.</i> , 1982)			+/-								
<i>Ae. vittatus</i>	(Cordellier <i>et al.</i> , 1974)	(Cornet <i>et al.</i> , 1979; Germain <i>et al.</i> , 1982)											
<i>Ae. fluviatilis</i>		(Lhuillier <i>et al.</i> , 1981)											
<i>Ae. scapularis</i>		(Lhuillier <i>et al.</i> , 1981)											
<i>Ae. simpsoni</i> group	(Cordellier <i>et al.</i> , 1974; Vainio and Cutts, 1998; Jupp and Kemp, 2002)	(Cordellier <i>et al.</i> , 1974)			+/-								
<i>Ae. dentatus</i>		(Cordellier <i>et al.</i> , 1974)											
<i>Ae. metallicus</i>	(Cordellier <i>et</i>				+/-								

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Species	Yellow fever		Cycle <sup>1</sup>		Habits	Distribution							
			Dom	Sylv	Anthr	Afr		Asia		Oc	Am		Eur
	Lab studies	Field isolation				W	E	ME	SE		SC	N	
	<i>al.</i> , 1974)												
<i>Ae. pseudoafricanus</i>	(Cordellier <i>et al.</i> , 1974)												

<i>Cx. thalassius</i>	(Cordellier <i>et al.</i> , 1974)												
<i>Cx. quinquefasciatus</i>	(Cordellier <i>et al.</i> , 1974)												
<i>Hg. leucocelaenus</i>	(Lhuillier <i>et al.</i> , 1981; Vainio and Cutts, 1998)												
<i>Hg. equinus</i>	(Lhuillier <i>et al.</i> , 1981; Vainio and Cutts, 1998)												

<sup>1</sup>Dom: domestic, Sylv.: sylvatic, Anthr.: anthropophilic, Afr.: Africa, Oc.: Oceania, Am.: America, Eur.: Europe, W: West, E: East, M-E: Middle-East, S-E: South-East, S-C: South and Central, N: North, df : domestic form.

Usually, human infections with YF virus in sylvatic cycles are incidentally and produced when people work or inhabit in the forest. Then, as in Africa, viremic man introduce YF virus into urban areas infested with *Aedes aegypti*.

The main urban YF vector in both continents South America and Africa is *Aedes aegypti* that transmits YF between humans. Laboratory competence assays have demonstrated that Brazilian populations of *Aedes aegypti* (Johnson *et al.*, 2002) and Brazilian and North American populations of *Ae. albopictus* (Lourenco de Oliveira *et al.*, 2003) became infected after a viraemic bloodmeal. In regions where *Aedes aegypti* and *Ae. albopictus* overlap, *Aedes aegypti* is more abundant in urban environment, while *Ae. albopictus* predominates in semi-rural localities and periphery of cities. Crossing this information, it is assumed that *Ae. albopictus* can link both the urban cycle (transmitted by *Aedes aegypti*) and the sylvatic cycle (maintained by *Haemogogus* mosquitoes)

Indirect evidence for vertical transmission of YF virus was provided in field studies when the virus was isolated from collected males of the *Aedes furcifer-taylori* group in eastern Senegal (Cornet *et al.*, 1979), and in wild males and recently emerged adults from field larvae of *Aedes aegypti*, the epidemic vector (Fontenille *et al.*, 1997). In South America, transovarian transmission was demonstrated in field when YF virus was isolated from nulliparous *Hg. janthinomys* during an important epidemic in the Amazon Brazil in 1998 (Mondet *et al.*, 2002). Laboratory assays also

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have demonstrated experimentally YF virus trans-ovarian transmission in other species as *Ae. aegypti* (Aitken *et al.*, 1979), *Ae. mascariensis* (Beaty *et al.*, 1980), *Haemagogus equines* in South America (Dutary and Leduc, 1981) and YF virus has been isolated from ticks. A first YF strain was isolated in *Amblyomma variegatum* adult males and eggs. Further isolations were performed from larvae emerged from the same egg batch and from *Cercopithecus* monkey on which larvae of the same batch have been fed (Cornet *et al.*, 1978; Saluzzo *et al.*, 1980; Cornet *et al.*, 1982). Ticks can facilitate the maintenance and survival of YF virus during the dry season being efficient virus reservoirs.

Few YF cases have been documented in Europe always associated to travellers returning from endemic regions. In November 2001 an imported case of YF into Belgium in a non vaccinated traveller returning from Gambia was documented by WHO. The patient died few days later. ([http://www.who.int/csr/don/2001\\_11\\_12/en/index.html](http://www.who.int/csr/don/2001_11_12/en/index.html)). In February 2000, WHO documented another non vaccinated traveller that became ill in Netherlands after returning from Suriname. ([http://www.who.int/csr/don/2000\\_02\\_25/en/index.html](http://www.who.int/csr/don/2000_02_25/en/index.html)). In August 1999, a German traveller returning from Cote d'Ivoire was diagnosed as YF. The patient died few days later in Berlin. (Promed Archive number 19990806.1355). This cases is only a sample of the total number detected.

Summary as provided by the authors:

- In tropical regions the virus still affects about 200,000 people annually in tropical regions of Africa and South America and is a threat for unvaccinated travellers going to these areas. Unimmunized travellers can import YF into areas with *Aedes aegypti* populations. The Caribbean, Central and North America as well as Asia and some Mediterranean regions (especially Middle East) are under risk of YF virus introduction and dissemination due to the increase of distribution and density of *Aedes aegypti* populations and the increase in international travel including adventure travel to remote and tropical areas. Europe as other areas still must be considered receptive areas, as they were subject to introduction and spread at the beginning of the past century.
- In addition, studies comparing competence for YF in strains of American *Aedes aegypti* and *Aedes albopictus* has demonstrated that the Asian mosquito tiger although less efficiently is capable of transmitting the virus. This is a worrying fact as many European countries have well established populations of *Ae. albopictus* and scattered introductions of YF are reported in Europe.
- The demonstration of vertical transmission in several field vectors, mainly in *Aedes aegypti* shows that the vector can keep the virus for long periods being the mosquito the real virus reservoir. This can lead to YF persistence in an area inside infected eggs in peridomestic breeding sites until the next favourable weather conditions. Another epidemiological implication is that YF can be transmitted few days after the emergence of new *Ae. aegypti* females in their first blood meal avoiding the extrinsic incubation period, being the transmission



in human population more frequent than for diseases restricted exclusively to horizontal transmission.

### 1.3.3. Chikungunya fever

Chikungunya is an arboviral disease transmitted by *Aedes* mosquitoes. Chikungunya virus is a member of the genus *Alphavirus* in the *Togaviridae* family. To date, different evolutionary lineages separated by geography have been identified corresponding to West African, South/East African and Asian (Powers *et al.*, 2000).

Chikungunya virus causes an acute illness similar to that seen in dengue and usually includes fever, rash and an incapacitating arthralgia, being the last one useful to differentiate Chikungunya illness from dengue. The illness typically begins with a sudden onset of fever preceding the rash and joint pain. Fever can last up to 10 days and the non pruritic rash begins 2-5 dpi and is distributed on the face, limbs and trunk of the body. The severe joint pain is the most significant symptom of Chikungunya virus disease, usually reporting patients with incapacitating pain that lasts for several weeks or months. Most infections are resolved in several weeks or months but some patients have developed chronic joint problems (cited in Powers, 2000)

A commercial vaccine against Chikungunya virus is still lacking, however promising results have been generated in previous assays from both phase 1 and 2 clinical trials (Edelman *et al.*, 2000). The lack of specific antiviral drugs for Chikungunya virus limits the treatment to the mitigation of symptoms, based on administration of analgesics, anti-inflammatory agents and antipyretics.

The virus was first isolated in Tanzania in 1953 during an epidemic of dengue-like illness. Since then, sporadic cases have been reported from several African countries, the Indian subcontinent and South East Asia (Table 11) (Enserink, 2006; Pialoux *et al.*, 2007). The vast scale of the recent outbreaks in the 2005-2007 epidemics in Indian Ocean Islands and India are of unprecedented magnitude and underlines the poor knowledge acquired about the biology of this virus (Mishra, 2006). Chronologically, these outbreaks began in Kenya 2004, and in 2005 new outbreaks were reported from the Comoros Islands, La Réunion, and other islands in the southwest Indian Ocean. At the same time another large outbreak appeared in India with estimations of more than 1,400,000 cases occurred since December 2005 (Pialoux, 2007)

In tropical regions of Africa and Asia Chikungunya virus appears to be enzootic. Chikungunya virus is zoonotic, and can be presented in both endemic and epidemic forms. The endemic form is characteristic from rural Africa being incriminated a wide range of sylvatic vectors and reservoirs, mainly *Aedes* mosquitoes and non-human primates, with continued transmission within immune population and sporadic small outbreaks. The epidemic form is typical from urban Asia and is transmitted by *Ae. aegypti* and *Ae. albopictus*. This form produces massive epidemics in population with weak herd immunity and can be sustained strictly by human-mosquito cycles in urban areas.

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Table 11. Vector competence studies and field isolation with Chikungunya virus

Species	Laboratory studies	Field isolation
<i>Aedes aegypti</i>	(Shah <i>et al.</i> , 1964 ; Jupp and McIntosh, 1990 ; Tsetsarkin <i>et al.</i> , 2007)	
<i>Aedes albopictus</i>	(Shah <i>et al.</i> , 1964 ; Jupp and McIntosh, 1990 ; Tsetsarkin <i>et al.</i> , 2007; Vazeille <i>et al.</i> , 2008 )	
<i>Aedes polynesiensis</i>	(Shah <i>et al.</i> , 1964)	
<i>Aedes furcifer</i>	(Paterson and McIntosh, 1964; Jupp <i>et al.</i> , 1981 )	(Diallo <i>et al.</i> , 1999)
<i>Aedes taylori</i>	(Paterson and McIntosh, 1964)	(Diallo <i>et al.</i> , 1999)
<i>Aedes luteocephalus</i>		(Diallo <i>et al.</i> , 1999)
<i>Aedes africanus</i>		(Diallo <i>et al.</i> , 1999)
<i>Aedes neoaffricanus</i>		(Diallo <i>et al.</i> , 1999)
<i>Aedes dalzieli</i>		(Diallo <i>et al.</i> , 1999)
<i>Aedes argenteopunctatus</i>		(Diallo <i>et al.</i> , 1999)
<i>Aedes vittatus</i>	(Mourya and Banerjee, 1987 ; Jupp and McIntosh, 1990 ; Vazeille <i>et al.</i> , 2008)	(Diallo <i>et al.</i> , 1999)
<i>Aedes fulgens</i>	(Jupp <i>et al.</i> , 1981 ; Jupp and McIntosh, 1990)	
<i>Aedes ledegeri</i>	(Jupp and McIntosh, 1990)	
<i>Aedes metalicus</i>	(Jupp and McIntosh, 1990)	
<i>Aedes caspius</i>	(Vazeille <i>et al.</i> , 2008)	
<i>Aedes detritus</i>	(Vazeille <i>et al.</i> , 2008)	
<i>Anopheles coustani</i>		(Diallo <i>et al.</i> , 1999)
<i>Anopheles rufipes</i>		(Diallo <i>et al.</i> , 1999)
<i>Aopheles. stephensi</i>	(Yadav <i>et al.</i> , 2003)	
<i>Culex ethiopicus</i>		(Diallo <i>et al.</i> , 1999)
<i>Culex thalassius</i>		
<i>Mansonia africana</i>	(Jupp <i>et al.</i> , 1981)	

In Africa, Chikungunya virus is maintained mainly in a **sylvatic cycle** involving wild non-human primates and *Aedes* mosquitoes. Species involved in sylvatic cycle are *Ae. furcifer*, *Ae. taylori*, *Ae. luteocephalus*, *Ae. africanus*, and *Ae. neoaffricanus* (McIntosh *et al.*, 1977; Jupp and McIntosh, 1988; Diallo *et al.*, 1999). In rural areas heavy rainfalls increases the abundance of sylvatic



mosquitoes a phenomenon that usually precedes outbreaks. The main vectors of Chikungunya virus epidemics in these regions are females of the *Ae. fuscifer-taylori* group (Jupp and McIntosh, 1990). Both the detection of Chikungunya specific antibodies in rodents and birds, as well as the of Chikungunya virus isolates obtained from squirrel, chiroptera and ticks, support the presence of secondary sylvatic cycles that contribute to maintain the virus in endemic areas when non-human primates are immunologically protected ((Diallo *et al.*, 1999)).

In Asia Chikungunya virus is maintained mainly in an **urban cycle** and, until recently, the main vector has been the urban mosquito *Ae. aegypti*. This antropophilic mosquito in close association with humans has been responsible for regional large outbreaks. Other common peridomestic species, as *Ae. albopictus*, *Ae. vittatus* and *Anopheles stephensi* have been found to be competent for Chikungunya virus in laboratory assays and their abundance has been confirmed in endemic areas (Table 11) (Soekiman *et al.*, 1986a, b; Mourya, 1987; Mourya & Banerjee, 1987; Turell *et al.*, 1992; Yadav *et al.*, 2003b).

In 2004 *Ae. aegypti* was the vector of Chikungunya virus in the epidemic of Kenya and the following outbreaks of Comoros and Seychelles.. In 2005 further outbreaks were detected in Reunion and Mauritius Islands in areas where *Ae. aegypti* was absent or scarce and *Ae. albopictus* was abundant. In Reunion Island were approximately 266,000 cases, being affected the 34% of the total island population. Molecular virologists detected a new mutation in viral isolates from *Ae. albopictus* transmitted outbreaks in these Islands, and also from further outbreaks in Mayotte (2006; (Schuffenecker *et al.*, 2006)) and Madagascar (2007; (de Lamballerie *et al.*, 2008)). The mutation in the envelope protein gene (E1-A226A→E1-A226V) confers the virus an adaptation to *Ae. albopictus*, improving viral replication and transmission efficiency in this vector (Tsetsarkin *et al.*, 2007). A large chikungunya outbreak was recorded in India during 2006, officially with 1.39 million affected cases. The outbreak was first recorded in Andhra Pradesh and subsequently spread northwards as far as Delhi. In Europe despite several infected tourists returned from the Indian Ocean islands while the outbreaks were ongoing no outbreaks were detected (Hochedez *et al.*, 2006; Krastinova *et al.*, 2006; Parola *et al.*, 2006; Beltrame *et al.*, 2007)). Finally, in August 2007, the first documented Chikungunya outbreak on the European continent appeared when a sudden epidemic produced more than 200 victims in and around Ravenna, Italy (Rezza *et al.*, 2007). The index case was a man from Kerala, India (a Chikungunya epidemic area) who was visiting a relative in Casteglione di Cerva. After entomological investigations, Chikungunya virus was detected by PCR in *Aedes albopictus* trapped during the outbreak. The phylogenetic analysis showed that the strain isolated was similar to those of the Indian subcontinent (Central/east African genotype) and was sharing the same mutation (A226V) than the strains of the Indian Ocean islands adapted to *Aedes albopictus* (Rezza *et al.*, 2007)

Summary as provided by the authors:

- Available information and published literature about Chikungunya virus was relatively poor until the 2005-2007 epidemics. From literature, it can be concluded that two major vectors are involved in most outbreaks and large epidemics, *Aedes aegypti* and *Aedes albopictus*.

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- Recently, a new point mutation was identified from Chikungunya virus which confers the virus an increased fitness for infectivity, dissemination and transmission in *Aedes albopictus* (Tsetsarkin *et al.*, 2007).
- As seen in the Italian Chikungunya outbreak reported in Ravenna, the first documented outbreak in Europe, the most likely way of Chikungunya introduction and local transmission in European and other industrialized countries has been demonstrated to be linked with the establishment and spread of the Asian tiger mosquito *Ae. albopictus* (Aranda 2006, Gratz 2004, Knudsen 1996, Romi 2006, Schaffner 2000/04 citats en Tsetsarkin, 2007 #126), and the introduction of viraemic travelers from endemic Chikungunya areas. Just from February 2005 to April 2006 at least 340 confirmed cases were imported in different European countries (Depoortere and Coulombier, 2006).

## 1.4. WEST NILE FEVER AND JAPANESE ENCEPHALITIS

### 1.4.1. West Nile fever

West Nile virus (WNV) is a mosquito-borne virus belonging to the family *Flaviviridae* and is a member of the Japanese encephalitis complex. WNV is enzootic primarily amongst birds (Malkinson *et al.*, 2002) and appears less frequently in mammals, with equines and humans considered to be dead-end hosts (Deubel *et al.*, 2001). WNV is neuropathogenic for birds, horses and humans (Smithburn *et al.*, 1940) and is maintained in natural cycles between birds and ornithophilic mosquitoes, particularly *Culex* (*Culex*) species. Horses and humans are considered incidental hosts (Campbell *et al.*, 2002): WNV can cause encephalomyelitis in horses and in humans the illness, even if usually mild, may evolve into fatal encephalitis (Campbell *et al.*, 2002). WNV is widely distributed in Africa, the Middle East, and Eurasia and was introduced recently into North America (Hubalek, 2000; Marfin *et al.*, 2001; Petersen and Roehrig, 2001). A WNV human epidemic is ongoing in the USA since 1999, while limited outbreaks occurred sporadically in southern Europe following importation of the virus in infected birds migrating from Africa with subsequent involvement of local mosquito populations ((Murgue *et al.*, 2002). Since its detection in the United States in 1999, WNV has spread across 47 mainland states<sup>3</sup>, resulting in more than 27,000 cases with 1,086 deaths in humans (CDC, 2008).

WNV arrived in Canada in 2001 (Gancz *et al.*, 2004) reaching the countries of the Caribbean and Central America in 2001 (Dupuis *et al.*, 2003; Estrada-Franco *et al.*, 2003; Komar *et al.*, 2003; Quirin *et al.*, 2004; Cruz *et al.*, 2005; Lefrancois *et al.*, 2005; Mattar *et al.*, 2005; Morales-Betoulle *et al.*, 2006; Pupo *et al.*, 2006), and was detected in Argentina in 2006 (Morales *et al.*, 2006). It was transmitted also in several countries in continental Europe and in the Mediterranean Basin. Recent outbreaks causing human encephalitis have occurred in Algeria in 1994 (Murgue *et al.*, 2001), Romania in 1996–2000 (Tsai *et al.*, 1998), Czech Republic in 1997 (Murgue *et al.*, 2001), Russia in

<sup>3</sup> USGS (2004). United States Geological Survey. West Nile Virus Maps.  
<http://www.westnilemaps.usgs.gov/historical.html>



1999 (Lvov *et al.*, 2000) and Israel in 2000 (Weinberger *et al.*, 2001). Epizootics of WN disease in horses occurred in Morocco in 1996 (Murgue *et al.*, 2001) and in 2003 (Schuffenecker *et al.*, 2005), Italy in 1998 (Autorino *et al.*, 2002) and France in 2000, 2003, 2004 and 2006 (Zeller *et al.*, 2004; Durand *et al.*, 2005).

This *Flavivirus* was isolated from several mosquito species from different genera. According with some reviews, WNV was detected from at least 75 species (Higgs *et al.*, 2004) (table 12).



**Table 12.** Mosquito species from which West Nile virus has been detected (Hubalek and Halouzka, 1999; Higgs *et al.*, 2004)

<i>Aedes aegypti</i>	<i>Culex antennatus</i>
<i>Aedes africanus</i>	<i>Culex decens</i> group
<i>Aedes albocephalus</i>	<i>Culex erraticus</i>
<i>Aedes albopictus</i>	<i>Culex ethiopicus</i>
<i>Aedes albothorax</i>	<i>Culex guiarti</i>
<i>Aedes atlanticus</i>	<i>Culex modestus</i>
<i>Aedes atropalpus</i>	<i>Culex neavei</i>
<i>Aedes canadensis</i>	<i>Culex nigripalpus</i>
<i>Aedes cantans</i>	<i>Culex nigripes</i>
<i>Aedes cantator</i>	<i>Culex perexiguus</i>
<i>Aedes caspius</i>	<i>Culex perfuscus</i> group
<i>Aedes cinereus</i>	<i>Culex pipiens</i>
<i>Aedes circumluteolus</i>	<i>Culex poicilipes</i>
<i>Aedes excrucians</i>	<i>Culex pruina</i>
<i>Aedes japonicus</i>	<i>Culex quinquefasciatus</i>
<i>Aedes juppi</i> + <i>caballus</i>	<i>Culex restuans</i>
<i>Aedes madagascarensis</i>	<i>Culex salinarius</i>
<i>Aedes signifera</i>	<i>Culex scottii</i>
<i>Aedes sollicitans</i>	<i>Culex tarsalis</i>
<i>Aedes taeniorhynchus</i>	<i>Culex territans</i>
<i>Aedes triseriatus</i>	<i>Culex theileri</i>
<i>Aedes trivittatus</i>	<i>Culex tritaeniorhynchus</i>
<i>Aedes tormentor</i>	<i>Culex univittatus</i>
<i>Aedes vexans</i>	<i>Culex vishnui</i> group
	<i>Culex weschei</i>
<i>Aedomyia africana</i>	
<i>Anopheles atropos</i>	<i>Culiseta inornata</i>
<i>Anopheles barberi</i>	<i>Culiseta melanura</i>
<i>Anopheles brunnipes</i>	<i>Deinocerites cancer</i>
<i>Anopheles coustani</i>	
<i>Anopheles crucians/bradleyi</i>	<i>Mimomya hispida</i>
<i>Anopheles maculipalpis</i>	<i>Mimomya lacustris</i>
<i>Anopheles maculipennis</i>	<i>Mimomya splendens</i>
<i>Anopheles punctipennis</i>	
<i>Anopheles quadrimaculatus</i>	<i>Psorophora ciliata</i>
<i>Anopheles subpictus</i>	<i>Psorophora columbiae</i>
<i>Anopheles walkeri</i>	<i>Psorophora ferox</i>





*Coquillettidia metallica*  
*Coquillettidia microannulata*  
*Coquillettidia perturbans*  
*Coquillettidia richiardii*

*Uranotaenia sapphirina*

It is not possible to conclude the potential role of mosquito species from such a list, because: i) it is difficult, if not impossible, to find the original publications, describing the WNV isolations, as from *Aedes caspius* for instance, and ii) some of these isolations were obtained from mix of unfed and blood-fed females, as for *Coquillettidia metallica*<sup>4</sup> (Woodall *et al.*, 1961) or for *Anopheles maculipennis* (Filipe, 1972). Moreover, in the USA, a higher number of mosquito species have been found positive for WNV presence (WNV isolation, WNV RNA detection, or WNV antigen detection using a variety of diagnostic tests)<sup>5</sup>. But, from all mosquito pools found positive for WNV RNA detection in the USA from 2001 to 2004, 77% were obtained from only 5 species. These were all *Culex* (*Culex*) species, namely *Cx. quinquefasciatus* 29%, *Cx. pipiens* 26%, *Cx. restuans* 10%, *Cx. tarsalis* 9%, and *Cx. salinarius* 3% (Hayes *et al.*, 2005).

The transmission of WNV by mosquitoes was first demonstrated experimentally in 1943 in *Aedes albopictus* (Philip and Smadel, 1943). Vector competence for WNV is well documented for numerous *Culex* species in countries where WNV circulation has occurred: i) *Cx. antennatus*, *Cx. pipiens*, and *Cx. univittatus* in Egypt and Israel (Tahori *et al.*, 1955), ii) *Cx. quinquefasciatus* in India (Varma, 1960), iii) *Cx. univittatus*, *Cx. theileri*, *Cx. quinquefasciatus*, and *Cx. neavei* in South Africa (Jupp and McIntosh, 1970a; Jupp and McIntosh, 1970b; Jupp *et al.*, 1972; Jupp *et al.*, 1981; Jupp *et al.*, 1986), iv) *Cx. tritaeniorhynchus* in Pakistan (Hayes *et al.*, 1980). Moreover, since the introduction of WNV into the United States, numerous North American mosquito species, both *Culex* and *Aedes* species, have been tested for susceptibility to transmit WNV experimentally (Sardelis *et al.*, 2001; Goddard *et al.*, 2002; Turell *et al.*, 2005a). From this abundant literature, it could be stated that some *Culex* species are the most competent species for WNV such as *Cx. univittatus*, *Cx. tritaeniorhynchus*, *Cx. antennatus*, *Cx. theileri*, *Cx. neavei* or *Cx. modestus* (Balenghien, 2006). Most of the other *Culex* species could be considered moderate WNV vector, such as *Cx. pipiens*, *Cx. quinquefasciatus*, and all North American *Culex* species (Balenghien, 2006). Almost all *Aedes* species can be considered poor WNV vectors. Only *Ae. albopictus* and *Ae. trivittatus* may represent moderate vectors, as *Cx. pipiens*, and only *Ae. atropalpus* and *Ae. japonicus* seem to be better vectors than *Cx. pipiens*, even if, for these latter species, studies remain sparse (Balenghien, 2006).

<sup>4</sup> “It is therefore probable that the isolation was made from a fresh blood-meal, and not from the tissues of a mosquito that was maintaining the virus biologically” Woodall J.P., Gillett J.D., Corbet P.S., Weinbren M.P. and Williams M.C. (1961). The isolation of West Nile virus from the bird-biting mosquito *Mansonia metallica* in Uganda. *Annals of tropical medicine and parasitology*. **55**: 398-402..

<sup>5</sup> CDC (2007) Center for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, West Nile Virus, Entomology. <http://www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm>



Through the world, the main species involved in WNV transmission belong to the *Culex* genus: *Cx. univittatus*, *Cx. antennatus* and *Cx. pipiens* in Egypt (Taylor *et al.*, 1956), *Cx. univittatus*, and *Cx. pipiens* in Israel (Nir *et al.*, 1972), *Cx. univittatus* and *Cx. theileri* in South Africa (McIntosh *et al.*, 1967), *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* in India and Pakistan (Pavri and Singh, 1965; Akhter *et al.*, 1982), and, in the USA, *Cx. pipiens*, *Cx. restuans* and *Cx. salinarius* in the North-East, *Cx. quinquefasciatus* in the South, *Cx. nigripalpus* in the East and *Cx. tarsalis* in the West (Andreadis *et al.*, 2004; Reisen *et al.*, 2004; Hayes *et al.*, 2005; Lukacik *et al.*, 2006).

In Europe, *Ae. geniculatus*, *Ae. punctor*, and *An. plumbeus* have showed to be able to experimentally transmit WNV (Vermeil *et al.*, 1960), but there is no field evidence that these species are involved in WNV transmission. *Aedes caspius* has been reported naturally infected with WNV (Hubalek, 2000), but the original publication is not available, and this species may be considered as not competent for WNV (Balenghien *et al.*, 2008). WNV was isolated from *Cq. richiardii* (Berezin, 1971), which should be considered a suspected vector<sup>6</sup>. According to virus isolations and vector competence studies, *Ae. albopictus* and *Ae. vexans* should be considered potential WNV vectors, even if virus isolations are sparse for the first species, and even if the second species may be regarded as a poor experimental vector.

In Europe, *Cx. pipiens* was found naturally infected by WNV in Czech Republic (Hubalek *et al.*, 1998), in Portugal (Esteves *et al.*, 2005), in Romania (Savage *et al.*, 1999), and in Russia (Fyodorova *et al.*, 2006). The ability of this species to transmit WNV by bite has been regularly established in various countries (Tahori *et al.*, 1955; Hurlbut, 1956; Goddard *et al.*, 2002; Tiawsirisup *et al.*, 2005; Turell *et al.*, 2005a). European populations of *Cx. pipiens* could be considered moderate competent for WNV<sup>7</sup> (Balenghien *et al.*, 2008). *Culex pipiens* was involved in the Cerbaie-Fucecchio outbreak (Autorino *et al.*, 2002) and in Romania, where it acted as both enzootic and epizootic vector (Savage *et al.*, 1999). Generally, in dry areas of Europe (urban or suburban zones), it could play the main role in WNV transmission between birds and from birds to mammals (Balenghien *et al.*, 2006; Balenghien *et al.*, 2008). Nevertheless, to define the possible role of *Cx. pipiens* in the maintenance and transmission of *arboviruses* it is necessary to deeply understand the bionomics of the various mosquito biotypes, which could play different roles and that cannot be distinguished morphologically (Petrarca *et al.*, 1980; Vinogradova, 2000).

*Culex modestus* was found naturally infected with WNV in southern France in the 1960s (Hannoun *et al.*, 1964) and more recently in Russia (Fyodorova *et al.*, 2006). *Culex modestus* could be considered an extremely efficient laboratory WNV vector<sup>8</sup> (Balenghien *et al.*, 2007; Balenghien *et al.*, 2008). *Culex modestus* larvae can be found in semi-permanent reed marshes, irrigation canals, or rice fields (Rioux, 1958), and it is associated with wetland ecosystems (river deltas or floodplain

<sup>6</sup> WNV was isolated from *An. maculipennis*, but due to the presence of blood-fed females in tested pools Filipe A.R. (1972). Isolation in Portugal of West Nile virus from *Anopheles maculipennis* mosquitoes. *Acta virologica*. **16**(4): 361., this species could not be considered a potential vector.

<sup>7</sup> Disseminated infection and transmission rates were 38.5% and 15.8% Balenghien T., Vazeille M., Grandadam M., Schaffner F., Zeller H., Reiter P., Sabatier P., Fouque F. and Bicout D.J. (2008). Vector Competence of some French *Culex* and *Aedes* for West Nile Virus. *Vector borne and zoonotic diseases*. in press..

<sup>8</sup> Disseminated infection and transmission rates were 89.2% and 54.5% Ibid..



ecosystems) from Europe to India. Adults show very low dispersal ability in open lands and high densities of aggressive females remain localized in breeding and resting places: for instance, reed marshes and riverine forest (Mouchet *et al.*, 1970). Because *Cx. modestus* feeds mainly on birds (Balenghien *et al.*, 2006), it could thus be involved in very efficient rural WNV cycles between wetland birds in breeding or resting sites where mosquito and bird densities are high, such as reed marshes. In these sites, it could serve as both a maintenance vector (i.e., bird to bird) and an epidemic vector (i.e., bird to mammal) in Europe, because this species also feeds aggressively on humans and horses (Mouchet *et al.*, 1970; Balenghien *et al.*, 2006).

#### 1.4.2. Japanese encephalitis

Japanese encephalitis (JE) virus is a mosquito-borne zoonotic *Flavivirus*, which could be considered the most common cause of encephalitis worldwide. It infects a wide range of vertebrate species in an enzootic cycle primarily of large waterfowl birds and swine. Horses and humans are considered bystanders to this enzootic cycle and, once infected, dead-end hosts.

Observations of the first described large epidemic (Japan, 1924) and subsequent epidemics (between 1931 and 1948) of JE, suggested that it was spread by a mosquito vector (mainly rice field breeding mosquitoes from the genus *Culex*), and had a seasonal disease occurrence.

The principal vectors of JE virus is *Culex tritaeniorhynchus* and related species, and its major amplifier is swine. The mosquito vector was confirmed by virus isolation from *Culex tritaeniorhynchus* mosquitoes in 1938 [Mitamura, 1936 #279]. Animal-vector-human host transmission of JE virus was fairly described by Scherer and colleagues, who elucidated the transmission cycle of JE virus between viremic pigs and birds to man as an incidental dead-end host by the vector *Culex tritaeniorhynchus* mosquitoes (Buescher and Scherer, 1959; Buescher *et al.*, 1959a; Buescher *et al.*, 1959b; Scherer *et al.*, 1959a; Scherer *et al.*, 1959b; Scherer *et al.*, 1959c). JE virus was first isolated in 1934 from the brain of a fatal case of encephalitis (Mitamura *et al.*, 1936; Monath, 1988). This virus isolate was characterized as the prototype (Nakayama) strain of JE virus.

JE infection in humans can manifest in a spectrum of disease from asymptomatic infection or a mildly febrile symptomatic illness, to acute meningoencephalitis, with permanent neurological damage reported in 50% of recovered cases (Solomon *et al.*, 2000). Fatality rates are high (reaching up to 25%) in young children (Burke and Leake, 1988). JE is the most common cause of encephalitis in most of Asia; the JE virus is now endemic throughout much of Southeast Asia (Mackenzie *et al.*, 2001) and is responsible for over 50,000 clinical cases annually.

JE is distributed widely throughout Asia with reported cases in India, Nepal, Sri Lanka, Bangladesh, Burma, Laos, Cambodia, Vietnam, Thailand, Malaysia, Singapore, Taiwan, Philippines, Indonesia, China, Siberia, Korea, and Japan. Disease incidence in humans is seasonal and varies by country occurring from May to September in the temperate climates of Korea and Japan, April to October in the tropical countries of Southeast Asia such as Thailand, Cambodia and Vietnam, and from September to December in Nepal and Northern India. This is probably due to irrigation practices, ambient temperature and the migration pattern of susceptible birds (Tsai and Yu, 2000). Although



JE continues to spread in Asia, vaccination campaigns coupled with an improvement in living conditions have significantly reduced the impact of the disease in Japan.

The first severe outbreak of disease occurred in Japan in 1924 with 6,125 cases and 3,797 deaths (Rappleye, 1939). Epidemic JE occurred in Japan again in 1927, 1935, and 1948 with the last major outbreak occurring in 1968 (Kono and Kim, 1969; Okuno, 1978; Umenai *et al.*, 1985). Epidemic JE was subsequently reported in Korea in 1949 when 5,548 cases were recorded. Epidemics of JE occurred again in Korea in 1958 with 6,897 cases and 2,177 deaths (Pond *et al.*, 1954), and then persisted with 1 000 cases annually until 1969, when cases dropped to fewer than 100 per year (Kim, 1986). Epidemic JE occurred again in 1973 (769 cases) and in 1982 (2975 cases) (Umenai *et al.*, 1985; Kim, 1986).

JE has been recognized in China since 1940 with the virus isolated in 1949 (Beijing and P3 strains). JE was not recognized as a public health problem until 1966 when disease incidence peaked with over 40,000 cases reported throughout the country (Chu *et al.*, 1940; Grayston *et al.*, 1962; Mackenzie, 1982).

In tropical southeast Asia and Asia, JE has been recognized as an endemic disease with periodic epidemics since the 1950s in Thailand, Vietnam, Burma, Bangladesh, Indonesia, Malaysia, the Philippines, Sri Lanka, India, and Taiwan (Paterson *et al.*, 1952; Grayston *et al.*, 1962; Carey *et al.*, 1968; Ketel and Ognibene, 1971; Ming *et al.*, 1977; Khan *et al.*, 1981; Burke *et al.*, 1985). In Cambodia, it is estimated that 18% of children hospitalised with clinical encephalitis is caused by JE.

JE was noted in southern India as early as 1954. The first major epidemic occurred in the state of West Bengal during 1977, and was followed by epidemics in Kolar and Karnataka in 1977 (Bu'Lock, 1986). In 1978, 1,256 cases were reported across central and eastern India with 544 deaths.

JE is now thought to be the most common cause of encephalitis in the Tarai region of Nepal and is a growing public health concern, with over 11.5 million people at risk (Henderson *et al.*, 1983; Khatri *et al.*, 1983; Bista *et al.*, 1999). From 1993 to 1997, the total cases of JE within 25 districts in the Tarai increased from 446 cases in 1993 to 2,953 in 1997 (Bista *et al.*, 1999).

Recently the south-eastern limit of JE activity was extended into Australia with the detection of human infections in 1995 to 1998 in the Torres Strait Islands and mainland Queensland, Australia (Hanna *et al.*, 1996; Hanna *et al.*, 1999; Williams *et al.*, 2000).

The eastern limit of JE activity has previously been described as bounded by the faunal boundary, Wallace's Line<sup>9</sup> (Burke and Leake, 1988). The eastern and southern regions of India define the western limit of JE.

JE virus has been isolated from a large number of mosquito species in field studies: in Malaysia from *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Mansonia* spp., *Aedes curtipes*, and mixed *Anopheles* spp.

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<sup>9</sup> Wallace's line is a hypothetical boundary between the Oriental and Australasian faunal regions proposed in the nineteenth century by the naturalist Alfred Russell Wallace. Wallace's Line extends south to north from the Indian Ocean through the Lombok Strait (between Borneo and the Celebes) and eastward, south of Mindanao, into the Philippine Sea. Wallace's Line represents an abrupt limit of distribution for many major animal groups including birds





(Simpson *et al.*, 1970; Simpson *et al.*, 1974), in southern India from *Cx. pseudovishnui*, *Cx. vishnui*, *Ae. subpictus*, in Pakistan from *Anopheles hyrcanus* group and *An. barbirostris*, in China from *Ae. chemulpoensis*, *Cx. pipiens* var. *pallens*, *An. hyrcanus*, *Ae. albopictus*, and *Armigeres obturans*, in Sri Lanka from *Cx. fuscocephala*, *Cx. whitmorei*, and *Mansonia uniformis* (Okuno *et al.*, 1973; Huang, 1982; George *et al.*, 1987; Peiris *et al.*, 1992; Gajanana *et al.*, 1997), and in northern Australia from *Cx. annulirostris* (Ritchie *et al.*, 1997; Johansen *et al.*, 2000; Johansen *et al.*, 2001) and *Cx. sitiens* (Vythilingam *et al.*, 1994; Vythilingam *et al.*, 1995).

Under laboratory conditions (vector competence experiments) it has been demonstrated that JE virus can infect and be transmitted by a large number of mosquito species; *Cx. tritaeniorhynchus*, *Cx. elidus*, *Cx. vishnui*, *Cx. pseudovishnui*, and *Cx. fuscocephala* are considered highly competent vectors in transmitting JE virus. *Culex pipiens pallens*, *Cx. quinquefasciatus*, *Cx. p. pipiens* biotype *molestus*, *Cx. tarsalis*, *Cx. pseudovishnui*, and *Anopheles tessalatus* are considered moderate competent vectors for JE viruses (Gresser *et al.*, 1958; Mourya *et al.*, 1991). *Aedes* spp. are considered to be the lowly efficient for JE virus transmission under laboratory conditions. Finally, *Cx. sitiens* and *Cx. annulirostris* are considered to be the most important vector of JE virus in the Australian region.

There are four important mechanisms for the environmental maintenance of JE virus in mosquitoes. The overwintering of JE virus in *Cx. tritaeniorhynchus* and *Cx. pipiens* has been demonstrated and may contribute to the environmental maintenance of this virus (Lee, 1971; Hayashi *et al.*, 1975; Ura, 1976). Transovarial transmission of JE virus can occur in *Cx. tritaeniorhynchus*, *Cx. bitaeniorhynchus*, *Cx. vishnui*, *Ae. albopictus*, *Ae. togoi*, and *Ae. aegypti* mosquitoes. JE virus transmission to the larval stage has been demonstrated to occur in *Cx. pipiens*, *Ae. vexans*, *Ae. alcasidi*, and *Ar. flavus* (Rosen *et al.*, 1989). For *Cx. vishnui*, 10% of female mosquitoes passed JE virus to the adults of the second generation (Soman *et al.*, 1986). In *Cx. tritaeniorhynchus* mosquitoes, the percentage of parent females transmitting to their progeny ranged from 12% to 100%. In addition, progeny infection rates varied by the interval of time between parental infection and oviposition suggesting that vertical infection was not transovarial but rather occurred at oviposition. In addition, JE virus is transmitted sexually from male to female *Cx. tritaeniorhynchus* and *Cx. bitaeniorhynchus* (Rosen *et al.*, 1989; Mourya and Soman, 1999).

Summary as provided by the authors:

- *Cx. tritaeniorhynchus* is a very efficient vector for JE virus replication and transmission, and is thought to be the primary proven vector in JE virus transmission throughout most of Asia. According with virus isolation from field-collected individuals and with vector competence studies, it could be stated that the others main (but regarded as secondary ones) JE virus vectors are: *Culex vishnui*, *Culex gelidus* and *Cx. annulirostris*.
- Based on available information and published literature, from all species potentially involved in JE virus transmission (virus isolation and study of vector competence), only *Aedes albopictus*



and *Cx. p. pipiens* biotype *molestus* are present in Europe and should be considered potential vectors even if *Aedes* spp. are considered to be the least efficient in JE virus transmission under laboratory conditions and *Cx. p. molestus* is competent in JE transmission but with lower efficiency.





## 1.5. CONCLUSION

Table 13 lists potential/suspected/proven vector species for some arboviroses present in Europe.

**Table 13.** Potential/suspected/proven vector species for some arboviroses in Europe

Species	Hazard for Europe	Vector status	Reference
<i>Culex pipiens</i>	Common in temperate countries	Proven vector of WN virus	(Tahori <i>et al.</i> , 1955; Hurlbut, 1956; Hubalek <i>et al.</i> , 1998; Savage <i>et al.</i> , 1999; Goddard <i>et al.</i> , 2002; Esteves <i>et al.</i> , 2005; Tiawsirisup <i>et al.</i> , 2005; Turell <i>et al.</i> , 2005a; Fyodorova <i>et al.</i> , 2006; Balenghien <i>et al.</i> , 2008)
		Potential vector of RVF virus (virus isolation, vector competence studies)	(Hoogstraal <i>et al.</i> , 1979; Meegan <i>et al.</i> , 1980; Turell <i>et al.</i> , 1996)
		Potential vector of JE virus (vector competent studies)	(Gresser <i>et al.</i> , 1958; Mourya <i>et al.</i> , 1991)
<i>Culex modestus</i>	Palearctic distribution	Proven vector of WN virus	(Hannoun <i>et al.</i> , 1964; Fyodorova <i>et al.</i> , 2006; Balenghien <i>et al.</i> , 2007; Balenghien <i>et al.</i> , 2008)
<i>Culex theileri</i>	Presence in some parts of Europe	Proven vector of WN virus in South Africa	(McIntosh <i>et al.</i> , 1967)
		Potential vector of RVF virus (isolation and laboratory studies)	(McIntosh, 1972; McIntosh <i>et al.</i> , 1973; McIntosh <i>et al.</i> , 1980)
Complex Univittatus	Presence in some parts of southern Europe	Proven vector of WN virus in Africa	(Taylor <i>et al.</i> , 1956)
		Suspected vector of RVF virus	(McIntosh <i>et al.</i> , 1980)
<i>Aedes albopictus</i>	Presence in some parts of Europe	Proven vector of dengue virus	(Vazeille <i>et al.</i> , 2003; Gratz, 2004)
		Suspected vector of VEE viruses (vector competence studies)	(Turell <i>et al.</i> , 1992; Fernandez <i>et al.</i> , 2003; Smith <i>et al.</i> , 2005)
		Potential vector of EEE virus (isolation and laboratory studies)	(Scott <i>et al.</i> , 1990; Mitchell <i>et al.</i> , 1992; Turell <i>et al.</i> , 1994)
		Potential vector of WN virus (isolation and laboratory studies)	(Tiawsirisup <i>et al.</i> , 2004; Hayes <i>et al.</i> , 2005; Tiawsirisup <i>et al.</i> , 2005)
		Suspected vector of JE virus (natural infection)	(George <i>et al.</i> , 1987)
<i>Aedes vittatus</i>	Western Mediterranean distribution	Potential vector of Dengue virus (experimental transmission, natural infection)	(Diallo <i>et al.</i> , 2003) (Mavale <i>et al.</i> , 1992)
<i>Aedes dorsalis</i>	European boreal distribution	Potential vector of WEE virus (experimental transmission, natural	(Spalatin <i>et al.</i> , 1963; Hayes <i>et al.</i> , 1976; Clark <i>et al.</i> , 1986; Fulhorst <i>et al.</i> , 1994;

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		infection)	Kramer <i>et al.</i> , 1998)
<i>Aedes vexans</i>	Whole Europe	Suspected vector of RVF virus (natural infection)	(Fontenille <i>et al.</i> , 1995; Jupp <i>et al.</i> , 2002)
<i>Aedes caspius</i>	Whole Europe	Potential vector of RVF virus (experimental transmission)	(Gad <i>et al.</i> , 1987; Turell <i>et al.</i> , 1996)



## 2. LITERATURE REVIEW FOR MOSQUITO SPECIES AS POTENTIAL/SUSPECTED/PROVEN VECTORS IN EUROPE

### 2.1. BIO-ECOLOGY OF POTENTIAL/SUSPECTED/PROVEN VECTORS IN EUROPE

#### 2.1.1. *Culex* species

##### ***Culex (Barraudius) modestus* Ficalbi, 1889**

*Culex modestus* is widely distributed in Europe and in the Central Asia as far as northern India (Gutsevich *et al.*, 1974).

**Bio-ecology.** The larvae of *Cx. modestus* are present from the beginning of spring throughout autumn (Mouchet *et al.*, 1970). Females overwinter within the vegetation (heaps of reeds) (Mouchet *et al.*, 1969). Adults are particularly present during the summer months and the beginning of autumn (Mouchet *et al.*, 1970). This species is autogenous (Schaffner *et al.*, 2001a).

The eggs are laid in rafts at the water surface, and hatch out quickly. Breeding sites occur in rural environments consist of permanent or partially permanent water receptacles. Larvae grow in rice fields, irrigation canals, and sem-permanent marshes. These breeding sites are generally very sunny and colonised by plants. Water can either be fresh or slightly brackish (less than 2 g/l of chlorides) (Schaffner *et al.*, 2001a).

Females are aggressive toward humans in the daytime but mainly at dusk or at night. They sometimes represent a serious nuisance for humans and other mammals, in the immediate surroundings of the larval sites. The females move about little (less than 1 km); they are not endophagous and even less endophilic (Schaffner *et al.*, 2001a).

**European distribution:** This Palearctic species is widely represented in wetlands of continental Europe, except for the most northern regions (Scandinavia and Baltic states).

##### ***Culex (Culex) pipiens* Linnaeus, 1758**

**Taxonomic status.** The great ecological plasticity of *Cx. pipiens* and its morphological and ethological variations have originated many descriptions of this taxon under very diverse names. The taxon is currently considered a plastic species with two forms in Europe: *Cx. pipiens* form *pipiens* and *Cx. pipiens* form *molestus* (Harbach *et al.*, 1985). Females of the typical form, *pipiens*, are rural, mostly bird-biting, they hibernate from autumn to spring, and they are anautogenous (they require blood-meal for laying the first egg batch) and eurygamous (they need open spaces for mating) (Vinogradova, 2000). Breeding sites consist of various types of pools and containers of water outdoor. On the other hand, females of the *molestus* biotype feed on humans in urban areas, they are formerly autogenous (they could produce the first egg batch without blood-meal) and stenogamous (they are able of mating in limited spaces), undergoing continuous generations during

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the whole year, without diapause (Vinogradova, 2000). Biological differences between the two forms may be associated with a rapid ecological adaptation of the *pipiens* form to the open aboveground rural breeding sites and of the *molestus* form to the closed underground urban breeding sites (Barr, 1981; Harbach *et al.*, 1984). Even if in northern Europe, both forms are well differentiated, and maybe in a speciation process (Rioux *et al.*, 1965), in southern Europe populations are clearly not genetically isolated (Rioux *et al.*, 1965; Pasteur *et al.*, 1981; Bourguet *et al.*, 1998; Chevillon *et al.*, 1998). Indeed, in southern Europe, *Cx. pipiens* populations have been described breeding in rural epigenous sites, stenogamous and anautogenous, with ornithophilic or anthropophilic preferences (Rioux *et al.*, 1965; Pasteur *et al.*, 1977; Balenghien *et al.*, 2008). The taxonomic status of *Cx. pipiens* is an endless debate (Fonseca *et al.*, 2004; Spielman *et al.*, 2004).

**Bio-ecology.** This multivoltine species is abundant during the summer and the autumn. The larvae appear toward mid-spring and disappear at the first frosts. The females overwinter in cellars, cowsheds, caves and other natural shelters (Vinogradova, 2000). In a hypogean environment, the species can continue to grow all year long laying autogenous eggs (Schaffner *et al.*, 2001a).

Larvae grow in waters very much polluted with organic matter (wastewater drainage ditches, temporary pond in the outskirts of cities, flooded ventilation space). They can also be found in sites where the water is cool and pure (drum containing rainwater, basin, banks of a non-polluted stream) (Schaffner *et al.*, 2001a). Larvae can be found in a wide range of breeding sites: natural or artificial, small or large water collections (Vinogradova, 2000).

Females bite all warm-vertebrates at night. In certain cities where wastewater is poorly managed, *Cx. pipiens* can be a nuisance of first importance. Host preferences of *Cx. pipiens*, especially determinants of mosquito choice between birds and mammals, are key factors of vector-borne transmission, such as West Nile fever.

**European distribution:** *Culex pipiens* is widely represented in the entire Holarctic region, and it is the most common species throughout Europe.

### ***Culex (Culex) perexiguus* Theobald, 1903**

**Taxonomic status.** *Culex perexiguus* is a member of the *Univittatus* complex, which includes two other species, *Cx. univittatus* and *Cx. naevy*. The specimens gathered in Europe and identified under the *Cx. univittatus* or *Cx. univittatus/perexiguus* form names in fact correspond to the *Cx. perexiguus* taxon (Schaffner *et al.*, 2001a).

**Bio-ecology.** *Cx. perexiguus* is abundant during the summer and the autumn. Larvae grow in many sorts of domestic (basin, wells, and drums) or natural (marshes, springs, drains, stream beds) breeding sites. The water in these sites is often clean and fresh but can sometimes contain a weak chloride concentration. Very little is known about the biology of the adults. Females seem feed mostly on birds; nevertheless they sometimes enter dwellings and bite humans at night (Schaffner *et al.*, 2001a).

**European distribution.** Its distribution area includes the arid areas of northern and eastern Africa, and south-western Asia. It is also present in several Mediterranean Europe countries (Spain, Portugal, Italy including Sicily, Macedonia, Greece, Turkey, Bulgaria, and maybe in Cyprus).



### ***Culex (Culex) theileri* Theobald, 1903**

**Bio-ecology.** *Culex theileri* displays 2 or 3 generations per year. The population density varies highly between different areas; the species is very much present during the summer and the autumn. It overwinters at the imago stage (Schaffner *et al.*, 2001a).

Larvae of this species can be found in great numbers of sites where the water is generally fesh but can also be slightly salted; the water may either be clean or polluted. The sites display abundant straight vegetation or not: ponds, marshes, rivers, reservoirs, residual puddles, springs, irrigation canals, and rice fields (Schaffner *et al.*, 2001a).

Females feed on all mammals. They generally bite outdoors, but can enter the dwellings to bite the humans; they are not considered as a major nuisance (Schaffner *et al.*, 2001a).

**European distribution.** The distribution area of this species is very large and includes the Afro-tropical region and widely encroaches up on the Palearctic and Oriental regions. It is present in the southern half of Europe (France including Corsica, Spain, Portugal, Slovakia, Italy including Sardinia and Sicily, Macedonia, Albania, Greece, Turkey, Hungary, Romania, Bulgaria, Moldavia, Ukraine, Byelorussia, European Russia).

### **2.1.2. *Aedes species***

#### ***Aedes (Stegomyia) albopictus* (Skuse, 1894)**

*Aedes albopictus* is also called Asian tiger mosquito because of its bright white straps. This species was originally indigenous to south-eastern Asia, inlands of the Western Pacific and Indian Ocean.

**Feeding habits.** This mosquito is an opportunistic blood feeder and has a broad host range, contrary to *Ae. aegypti aegypti* that feeds almost exclusively on humans. It can bite a wide variety of mammals, including cows and rats, as well as birds and reptiles. It has been documented that >92% of the field-collected *Ae. albopictus* females fed on blood of mammals (Savage *et al.*, 1993; Niebylski *et al.*, 1994). *Aedes albopictus* bites in the daytime, rarely at night. The time of peak biting activity varies with habitat, but most of the time a late afternoon peak was observed (Hawley, 1988). *Aedes albopictus* females have been collected biting both outdoors and indoors, but the comparative data available indicate that this is usually an outdoor biting mosquito (Hawley, 1988). More recently, this mosquito has developed behavioural changes that promote human biting, such endophagy and night-time biting (Drago, 2003).

At daytime, adults rest near the ground in undergrowth (Bohart, 1956). Adult flight range is quite short. Studies have shown that marked *Ae. albopictus* tend to remain in the area in which they are released and do not disperse more than several hundred of meters (Bonnet and Worcester, 1946). *Aedes albopictus* flies close to the ground and are not observed flying in strong winds (Bonnet and Worcester, 1946). Wind aid long distance dispersal would thus be unlikely to occur in this species. Therefore, most medium and long rang colonization is the result of passive transportation.





**Breeding sites.** *Aedes albopictus* is an opportunistic container breeder that is capable of utilizing natural as well as artificial container habitats. It has the ability to adapt to an exceptionally wide range of confined water sources. The mosquito is known for its ability to survive in very small collections of water, requiring only 1/4" of depth to complete its life cycle. In nature this species breeds in tree holes, bamboo stumps (formed by cutting cultivating bamboos), rock pools and other small natural reservoirs. It has easily adapted to human settlements. Its ecological flexibility allows it to colonise many types of human-made sites and urban regions. It may reproduce in flower pots, vases, bird baths, bucket cans, abandoned receptacles and especially used tires. All these receptacles can replace tree holes, provided there is a bit of vegetation nearby. The addition of decaying leaves from neighbouring trees produces chemical conditions similar to tree holes, which provides an excellent substrate for breeding.

*Aedes albopictus* eggs are able to withstand desiccation. The maximum longevity of an egg is 243 days (Gubler, 1970). Unlike *Ae. aegypti*, *Ae. albopictus* is able to introduce photoperiodic egg diapause, allowing overwintering in temperate regions (Hawley, 1988).

**Climatic factors** (source (2007; Straetemans, 2008)). Once *Aedes albopictus* is introduced to a specific area, the establishment of the vector depends on four main environmental factors:

- 1) Minimal winter temperature at which eggs will survive the winter. If winter temperatures drop below a certain temperature, the eggs will not survive. Areas with mean January temperature  $\geq 0^{\circ}\text{C}$  (conservative threshold, with the lowest threshold reported at  $-3^{\circ}\text{C}$ ) are generally accepted as overwintering areas (Medlock *et al.*, 2006);
- 2) Sufficient amounts of water to fill appropriate aquatic breeding sites of *Ae. albopictus*. An average annual rainfall of at least 500mm is required;
- 3) A sufficient amount of summer rainfall is necessary in order to maintain vector breeding places during the warm season;
- 4) Summer temperatures. Temperature influence the speed of development from the immature stages (larvae, pupae) to adult mosquitoes. The development rate is optimal when temperatures are between  $25^{\circ}\text{C}$  and  $30^{\circ}\text{C}$ . During summers with mean temperatures of over  $25^{\circ}\text{C}$ , the biological cycle from egg hatching to adult may be completed in 6–7 days. The speed of egg production after the female mosquito's blood meal (gonotrophic cycle) also increases with higher summer temperatures and hence summer temperatures are associated with increased abundance and a higher likelihood of the establishment of the mosquito.

**Recent spreading and present distribution.** *Aedes albopictus* was first described as the "banded mosquito of Bengal" by Skuse (1884) (Skuse, 1894) from Calcutta in India. This mosquito hails from East and Southeast Asia. It is believed to have spread along with humans to Madagascar and the smaller Indian Ocean Islands centuries ago. But its wide spreading came with the advent of modern shipping. Before 1979, *Ae. albopictus* was widely spread in an area from the Pacific to Madagascar and the Seychelles, in the Indian Ocean; in the north it was found in China, Korea and Japan (Huang, 1979b). In warmer area it was found throughout most of the South-east Asia region and west to Hawaii (Mitchell, 1995). The first modern establishment outside this original range occurred in 1979 in Albania, breeding in tyres thought to have been imported from China (Adhami and Murati, 1987). This was the first recorded infestation outside Oriental and Australasian regions





(Adhami and Reiter, 1998). *Ae. albopictus* was next detected in the United States in 1985 in Harris County, Texas (Sprenger and Wuithiranyagool, 1986). Although scattered individuals had already been sporadically collected in the country (Reiter, 1998), this was the first established population that was discovered. In the USA, the mosquito dispersed very rapidly throughout eastern and central USA and in 2001 it was found in California (Linthicum *et al.*, 2003), where it may have become established after arriving from China in a shipment of “Lucky bamboo” (*Dracaena sanderiana*), a popular decorative plant. In 2003, 866 counties in 26 states were infested (CDC, unpublished data, cited by (Eritja *et al.*, 2005)). In 1986 *Ae. albopictus* was detected in Brazil (Forattini, 1986) (Brito *et al.*, 1986). In 2002, the species was present in 20 of the 27 states of Brazil. (Santos, 2003). In 1988, *Ae. albopictus* was found breeding in a tyre close to the border of Texas. In 1993 the species has spread in two northern states of Mexico (Ibanez-Bernal and Martinez-Campos, 1994) and in a southern state (Casas-Martinez and Torres-Estrada, 2003). Between 1986 and 1995, the species was also detected in most of Central America (Honduras, Costa Rica, Guatemala, El Salvador, Panama), some of the Caribbean islands after 1993 (firstly Dominican Republic, then Cayman Islands and Cuba). More recently, *Ae. albopictus* has also been reported from Guatemala and Bolivia (1995), Colombia (1997), Argentina (1998) and Nicaragua (2003).

In the Pacific area, *Ae. albopictus* was detected in Salomon, Australia (1988), Fidji (1988), New Zealand (1994), and La Reunion (1994).

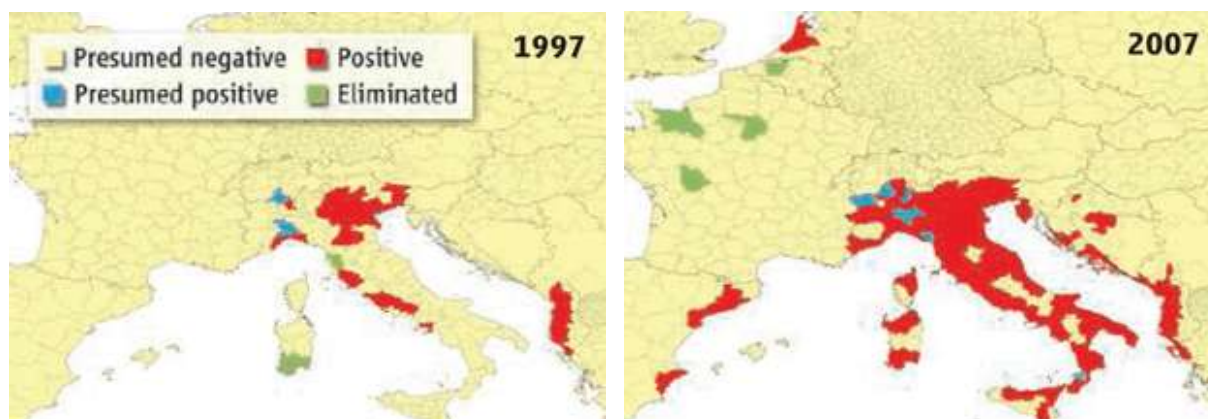
In 1990, living *Ae. albopictus* larvae have been found in South Africa, in tyres imported from Japan (Cornel and Hunt, 1991). Shortly thereafter, breeding populations were reported in Nigeria (Savage *et al.*, 1992). It was found to be well established in southern Cameroon (Fontenille and Toto, 2001). Populations have also been detected on Bioko Island (Equatorial Guinea) (Gratz, 2004). No other African country has reported *Ae. albopictus*, but the scarcity of surveys might mask a broader presence in the continent.

In Europe, in September 1990, an infestation of *Ae. albopictus* was discovered in the city of Genoa in Italy, in a school playground where many tyres had been left to be used by the children for play (Sabatini *et al.*, 1990). This mosquito has rapidly spread and was present in 9 regions in 2001 (Romi, 2001). In France, *Ae. albopictus* was first found in 1999 in two tyre dumps in France in 1999 during a specific survey (Schaffner *et al.*, 2001b). The presence of *Ae. albopictus* was detected the same year in a new continental location and in Corsica (Scholte and Schaffner, 2007). *Aedes albopictus* was discovered in the year 2000 in one location in Belgium, which became the fourth European positive country (Schaffner *et al.*, 2004).

As of 2007, the vector had been observed in Albania, Bosnia and Herzegovina, Croatia, France (Côte d’Azur and Corsica), Greece, Italy, Montenegro, Serbia, Slovenia, Spain and Switzerland (Scholte and Schaffner, 2007). In the Netherlands, where *Aedes albopictus* has only been found in greenhouses of companies importing lucky bamboo. Sporadically, adult mosquitoes have been found in the immediate surroundings of these greenhouses.

This species has also been reported from discarded tires in the vicinity of an airport in Israel (Pener *et al.*, 2003).

### Figure 1. Spread of *Ae. albopictus* in Europe



From E. J. Scholte, F. Schaffner, and P. Scarpulla  
*Emerging pests and vector-borne diseases in Europe*

**Specific surveillance system for *Ae. albopictus*** (source (2007; Straetemans, 2008)). There is currently no European standard for vector surveillance methods, which influences the comparability of data between countries. Different trapping techniques currently being used in Europe include oviposition traps (containers with water that allow female mosquitoes to lay eggs), carbon dioxide-baited counter flow traps (mosquito traps that utilise an outgoing airflow which carries chemical lures that attract mosquitoes to the trap and an incoming air flow draws mosquitoes into a collecting chamber), CDC traps (Centres for Disease Control and Prevention traps for adult mosquitoes, usually baited with carbon dioxide) and larval surveys (surveys to water collections, especially in containers to look for mosquito larvae). Furthermore, the density of the mosquito eggs in ovitraps does not necessarily reflect the density of the mosquito population in the field, as other breeding sites may be available; it gives an indication of the presence or absence of the vector in a certain area. Vector surveillance in member states of the European Union is mostly conducted in regions at high risk for establishment of *Ae. albopictus*. Based on local data, geographic areas likely for an introduction of *Ae. albopictus* can be mapped, e.g. storage centres for imported used tyres, main road axes or ports. Combining such maps with the risk of establishment once the vector has been introduced provides useful guidance on where to focus vector surveillance activities on national and local levels. Local characteristics and microclimates should be considered not only for the establishment of *Ae. albopictus*, but also for its likely abundance. These characteristics include urban vegetation, human population density and housing.

**Control of *Ae. albopictus*.** Once it has become established, it seems quite impossible to eradicate this mosquito (Reiter, 1998). The only option is to reduce vector densities to a level at which disease transmission is unlikely. However, this aim is difficult to achieve, the required measures are costly and trained vector control personnel will be needed.

Eliminating breeding sites, such as flower pots and vases, is effective, but requires the public participation, which is hard to sustain.

Spraying insecticide is another employed tactics, but its effectiveness is probably limited. Hiding in the vegetation, adult mosquitoes are hard to reach with aerosol droplets.

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Countries that have not yet seen the tiger mosquito can hope to prevent it from entering and can be hit hard if it does invade. But again, the options for prevention and control are limited. For its medium-distance travel, the mosquito has been known to travel in automobiles and trucks. By this way, it has apparently spread from Italy to Spain, France, Croatia, Slovenia, Switzerland, and Germany. At present it seems impossible to stop this type of spread.

To prevent long-distance infestations, governments would have to regulate the international tire trade. But as yet, only few governments have been willing to take measures that may affect the economy in this sector to thwart an uncertain public health risk. Another route of potential introduction that should be closely monitored is the trade with lucky bamboo.

### ***Aedes (Ochlerotatus) caspius caspius* (Pallas, 1771)**

**Taxonomic status.** *Aedes caspius* is a systematic complex. On one hand, the taxon includes 2 subspecies: *Ae. caspius meira*, endemic to the Cape Verde archipelago and *Ae. caspius caspius*, the latter being the sole representative in Europe. On the other hand, two divergent forms from a genetic point of view have been described under the *A* and *B* nomenclature. *Aedes caspius* can be mistaken with *Ae. dorsalis*, which morphologically very similar (Schaffner *et al.*, 2001a).

**Bio-ecology.** *Aedes caspius* overwinters at the egg stage. In the warm regions, the adults are present all year long but they are more abundant during spring after an increase in the water temperature and a lengthening of the photoperiod, which stop the egg diapause. The species is multivoltine and the bands follow one after the other at the same rhythm as the sites are flooded. The females produce a large quantity of eggs; the *A* species is potentially autogenous (Schaffner *et al.*, 2001a).

The exochorion of the eggs dorsally displays sub-lozenge polygons defining an area decorated with 14-16 small tubercles. They are laid individually, at the basis of vegetation tufts in sediments which are rich in organic matters but relatively poorly salted (4 to 7 g of chlorides p. 1,000). The eggs can be associated with those of *Ae. detritus* or *Ae. coluzzii* but the latter show a preference for more salted sediments. Several immersion and dessication cycles may be needed in order to induce the hatching of the eggs.

Larvae specially favour the coastal or continental brackish marshes, preferring the salted geological formations' outcrops. The breeding sites are very variable, more often large (ponds, marshes, rice fields, canals...) but sometimes smaller (ditches, deserted wells...). The species proliferates in brackish waters (1 to 30 g/l of chlorides), but may also be found in freshwater environments such as flooded meadows and rice fields. The presence of abundant vegetation is common. The postponed hatching of several eggs means that larvae can be present almost all year long in the breeding sites (Schaffner *et al.*, 2001a).

Females bite all warm-blooded vertebrates mostly outside the dwellings. Very anthropophilic, they are often responsible for strong nuisances, even in towns far removed from the larval sites. The imagoes can travel over 40 km to find their blood meal. A high number of control campaigns has particularly focused on this species (Spain, France, Italy, Greece...) (Schaffner *et al.*, 2001a).

**European distribution.** This Palearctic species, present throughout Europe, becomes scarcer towards the North. Up to now, the *A* form has been recorded in Continental Italy, Sardinia and France, and the *B* form only in Italy (Schaffner *et al.*, 2001a).



### ***Aedes (Ochlerotatus) dorsalis* (Meigen, 1830)**

**Taxonomic status.** For a long time considered as a sub-species of the *Ae. caspius* taxon, in particular by the Russian school, the validity of this species was confirmed by the evidencing of the reproductive isolation. It is quite possible that 2 different species are actually mingled under the *Ae. dorsalis* name: one would be present in the northern parts of the Holarctic region and correspond to the *dorsalis* type, and the other would be *Ae. albineus* Séguy 1923, found only in the desert regions of the South of the Palearctic region (Schaffner *et al.*, 2001a).

**Bio-ecology.** This species is multivoltine and the bans follow one after the other at the same rhythm as the sites are flooded. The larvae are present from the end of winter throughout the beginning of autumn. Adults appear toward mid-spring and disappear at the first frosts. The egg, the overwintering stage, initiates diapause at the end of summer, when the nycthemeron decreases. The species is autogenous (Schaffner *et al.*, 2001a).

The exochorion of the eggs dorsally displays hexagonal polygons defining an area decorated with 10-12 small tubercles. The eggs are laid in small groups; they are resistant to desiccation and frost. They hatch as soon as the sites are flooded. Larvae grow in ponds and marshes with more or less brackish water, generally shallow. These sites are most often near the coasts but also in the continental regions where salty grounds outcrop (Schaffner *et al.*, 2001a).

Females bite humans and all other mammals; they are aggressive during the day and even more before sunset, and can enter the dwellings. Carried by the wind, they can be a nuisance on several kilometres around the larval site. *Aedes dorsalis* is a very antropophilic species, which can pullulate because its larval sites are very large; the resulting nuisance is then very important for humans and other animals (Schaffner *et al.*, 2001a).

**European distribution.** *Aedes dorsalis*, a Holarctic circum-boreal species, is present in all northern Europe and becomes scarcer toward the South.

### ***Aedes (Aedimorphus) vexans vexans* (Meigen, 1830)**

**Taxonomic status.** The *Aedes vexans* taxon consists up of 3 sub-species: *Ae. vexans niponii* found in East Asia, *Ae. vexans arabiensis* found in Africa and *Ae. vexans vexans*, the latter being the only European representative.

**Bio-ecology.** *Aedes vexans* is multivoltine, in spring and summer. Larvae are found from the middle of spring to the end of summer, with an abundance peak from May to July. Imagos disappear during autumn. The egg is the overwintering stage.

After an incubation period of 4 to 10 days, the eggs are laid on the moist mud of draining ponds; they can stay on the dry soil during many years. If the water temperature (> 10°C) and light conditions are favourable, they will hatch synchronously when the breeding site is flooded. The eggs are also frost resistant. The development cycle is fast; it lasts 4 to 25 days depending on temperature and food abundance (Schaffner *et al.*, 2001a).

*Aedes vexans* is a typical flood-plain and delta mosquito (Danube, Rhine, and Rhone). Larvae generally breed in great quantities in flooding zones, with or without vegetation. It is found mostly





in flooded prairies, ditches, and ponds, most of the time in the company of *Ae. sticticus* (Schaffner *et al.*, 2001a).

During the day, imagos stay in the vegetation, shaded from the sun. At dusk, males gather in swarms, situated at the vertical of prominent objects, where females join them to find a mating partner. Mating is performed on vegetation. Good flyers, the adults travel great distances and a productive breeding site can create a nuisance up to 40 to 50 km away (Schaffner *et al.*, 2001a).

The females can live up to two months; they take their blood meal on numerous hosts. They bite, throughout the nycthemeral cycle, but particularly at dusk, humans as well as livestock and secondarily birds. *Aedes vexans* is one of the human biting *Aedes* the most widely spread in the world. Strongly aggressive and sometimes very abundant, this mosquito causes a strong nuisance in wetlands and rice fields in spring; it can enter houses to find its blood meal. Numerous control campaigns have this species as the prime target (Danube, Rhine, and Rhone valleys) (Schaffner *et al.*, 2001a).

**European distribution.** Its distribution area spreads to the Palearctic, Nearctic, Oriental and Australian regions. Found throughout Europe, it becomes sparser towards the South.

#### *Aedes (Fredwardsius) vittatus* (Bigot, 1861)

**Taxonomic status.** The species was recently transferred from the sub-genus *Stegomyia* to the new sub-genus *Fredwardsius*, due to important morphological differences with other species of the existent sub-genus (Schaffner *et al.*, 2001a).

**Bio-ecology.** *Aedes vittatus* is stenogamous and a summer species. The eggs are spindle-shaped. Under the microscope, the cells of the endochorion are polygonal, without any clear orientation. The eggs, very resistant to desiccation, are laid on the walls of the breeding sites, above the water. Their diapause is ended when they are rehydrated. They can be collected throughout the year in the dried mud of the breeding sites (Schaffner *et al.*, 2001a).

Larvae develop in rock holes and more rarely in containers. The water in breeding sites is fresh, sometimes brackish; the breeding sites are small, permanent or temporary, but always sunny. Larval development can be very fast if the water is warm and the food abundant; it can thus last from 7 to 12 days. The preimaginal stages can resist to temperatures above 40°C but die below 7°C (Schaffner *et al.*, 2001a).

This species is clearly stenogamous. Adults do not fly far from their breeding sites. The males are attracted by light. The females are exophilic and bite essentially during the day and at dusk. They feed on any available mammal (Schaffner *et al.*, 2001a).

**European distribution.** It is found in the Palearctic European sub-region, the Afro-tropical and the Oriental regions. In Europe, its distribution is restricted to the occidental Mediterranean region (France including Corsica, Spain including Balearic Islands, Portugal, and Italy including Sardinia and Sicily).



## 2.2. SURVEILLANCE SYSTEM OF MOSQUITOES IN EUROPE

The aims of entomological surveillance are: i) detect presence of a vector species, surveillance of the introduction of an exotic species, ii) follow spatial and temporal dynamics of species under interest, and/or iii) assess the abundance of a vector to determine a risk of transmission.

Methods for collecting mosquitoes are various depending on the aim. The most widespread are, for adults, CDC traps (Centres for Disease Control and Prevention), usually baited with light or carbon dioxide. For specific purposes, baited traps (as bird-baited traps (Lepore *et al.*, 2004)) or female gravid traps (increase the chance to detect virus infected females (Reiter *et al.*, 1986; Reiter, 1987)) could be used in surveillance programs. Moreover, mainly for *Ae. albopictus* which is poorly collected with classical CO<sub>2</sub> traps (Gingrich and Williams, 2005), oviposition traps (containers with water that allow female mosquitoes to lay eggs) or sticky traps (same oviposition traps but with sticky screen collected laying females) have been developed (Facchinelli *et al.*, 2007). The main difficulty in surveillance system using adult traps is to link the number of adults collected to the “real” abundance and to the transmission risk. For instance, the density of the mosquito eggs in oviposition traps does not necessarily reflect the density of the mosquito population in the field, as other breeding sites are available; it gives an indication of the presence or absence of the vector in a certain area (2007; Straetemans, 2008). Network of oviposition traps is currently used in east-southern France to monitor the establishment and the extension of *Ae. albopictus* from Italy.

Larval surveys (surveys to water collections, especially in containers to look for mosquito larvae) could be used in surveillance programs. For instance, several indices have been described and are currently used to monitor *Ae. aegypti* populations for dengue virus transmission. Those related to immature populations include the house index, i.e. the percentage of houses infested with larvae and pupae; the container index, i.e. the percentage of water-holding containers infested with larvae or pupae; and the Breteau index, i.e. the number of positive containers per 100 houses inspected. These indices could be used to assess a risk transmission or the efficiency of a control program.

## 2.3. MOSQUITO CONTROL IN EUROPE

Mosquito control can be carried out for vector control, or for nuisance control. In Europe, control is mainly focused on mosquito pests such as *Culex pipiens* in large cities, *Aedes caspius*, *Aedes vexans* and *Aedes detritus* in littoral areas or river plains. Nevertheless, the recent outbreak of Chikungunya fever in Italy underlines the possible return of vector control in Europe.

European authorities and public operators involved in mosquito control and the management of natural areas are grouped in the EDEN association<sup>10</sup>. It was founded on 4 May 1999 in Barcelona. It is an international non-profit making association subject to Belgian law with members from French, Spanish, Greece, and German regional public authorities and organizations. The aim of EDEN is to promote: regional solidarity, regional development, and wetland management, all of which relate to the policies agreed on by the authorities that manage or commission these environmental operators.

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<sup>10</sup> [http://www.eid-med.org/fr/Site\\_Eden/EDEN\\_e/accueil\\_e/accueil\\_e.htm](http://www.eid-med.org/fr/Site_Eden/EDEN_e/accueil_e/accueil_e.htm)





Currently, most of the European control programs focus on floodwater *Aedes*. For these species, the sites where females lay their eggs can be characterized by phyto-sociological analyzes, and thus it is possible to establish ecological maps of suitable breeding sites to focus geographically the control (Rioux *et al.*, 1968; Piakis *et al.*, 2007). These areas are sprayed with insecticides just after the flooding of the breeding sites to target the mosquito populations as larvae (Piakis *et al.*, 2007). Similar insecticides were used to treat mosquitoes in the different European member countries. Proportions differed depending on the environment and all products are certified by the various Ministries of Agriculture. The main anti-larvae products included organophosphates (temephos), and biological insecticides (*Bacillus Thuringiensis* serotype *israelensis* BTI and *Bacillus Sphaericus*).

The 98/8/EC directive (16 February 1998), relating to biocide products decreed new rules governing the authorization of commercially available products. To be authorized, the active products have to be reviewed for biological, toxicological, and ecotoxicological characteristics in the framework of the 91/414/EC European directive (15 July 1991), after that manufacturer required drawing up a report to defend these active ingredients. Due to these changes in European regulations, organophosphates have not been used as anti-larval in mosquito control for nuisance since the 1<sup>st</sup> of December 2006.

Secondarily, mosquito control programs focus on urban or peri-urban mosquito pests. For control of mosquitoes such as *Cx. pipiens*, which could cause nuisance in large cities, water management remains the key factor for durable control (Rioux *et al.*, 1965). For mosquitoes breeding in multiple small containers such as *Ae. albopictus*, the control consist to eliminate breeding sites, such as flower pots and vases, is effective, but it requires the public participation, which is hard to sustain. Sterile insect techniques (SIT) are currently under development for the control of *Ae. albopictus* in Italy (Bellini *et al.*, 2007).



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